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REQUEST FOR PRIORITY

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In accordance with the provisions of 37 CFR §1.55 and the requirements of 35 U.S.C. §119, filed herewith a certified copy of:

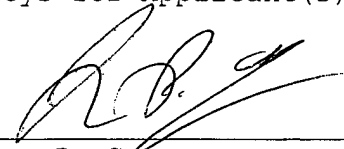
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It is respectfully requested that applicant be granted the benefit of the priority date of the foreign application.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By


Iver P. Cooper
Registration No. 28,005

IPC:jmb
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
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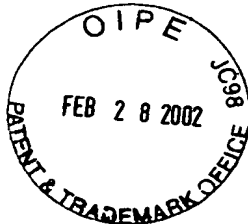
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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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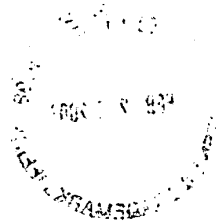
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Anmelder:
Applicant(s):
Demandeur(s):
Lannfelt, Lars
116 43 Stockholm
SWEDEN
Nilsberth, Camilla
115 59 Stockholm
SWEDEN

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Further applicants:
Anita Westlind-Danielsson, Storsvängen 159, 129 44 Hägersten, Sweden;
Jan Bäslund, Furusundsgatan 12, 11537 Stockholm, Sweden.

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Mutation APP for diagnosing and modelling Alzheimer's disease

The present invention relates to the use of an isolated nucleic acid characteristic of human amyloid precursor protein (APP) 770 including a glycine substitution at codon 693, that
5 corresponds to arctic mutation APP, for diagnosing or predicting a predisposition to Alzheimer's disease (AD) in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said arctic mutation, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, and by
10 accelerated formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) compared to protofibril formation of wild type A β peptides.

Also provided is a polypeptide corresponding to the arctic mutation APP and antibodies generated against said polypeptide. Further, the present invention provides a transgenic non-human mammal comprising a gene encoding amyloid precursor protein mutated at codon 693 in such a way that this codon encodes glycine. Finally, the present invention
15 leads to possible therapeutic intervention using drugs targeted at preventing protofibril formation.

Background of the invention

20 Alzheimer's disease (AD) is a progressive disease known generally as senile dementia. The disease falls into two categories, namely late onset and early onset. Late onset, which occurs in old age (65+ years), may be caused by the natural atrophy of the brain occurring at a faster rate and to a more severe degree than normal. Early onset AD is much more infrequent but shows a pathologically identical dementia with diffuse brain atrophy which
25 develops well before the senile period, i.e. between the ages of 35 and 60 years. One form of this AD type runs in families and it is known as familial Alzheimer's disease (AD).

Both types of AD are characterized by two types of lesions in the brain: senile plaques and neurofibrillary tangles. Senile plaques are areas of disorganized neuropil up to 150 mm
30 across with extracellular amyloid deposits at the center. Neurofibrillary tangles are intracellular deposits of amyloid protein consisting of two filaments twisted about each other in pairs.

The major protein subunit, A β (also referred to as amyloid (3 protein (A β P), (β -amyloid protein and A4) of the amyloid filaments of both the neurofibrillary tangle and the senile plaque, is a highly aggregating small polypeptide having a molecular weight of approximately 4,500. This protein is a cleavage product of a much larger precursor protein referred to as amyloid precursor protein (APP).

The sequence of the deposited A β in particular brain regions is one of the main pathologic characteristics of AD. The A β protein comprises 39 - 42 amino acids, and it is derived, as an internal cleavage product, from one or more isoforms of a larger APP. There are at least five distinct isoforms of APP: 563, 695, 714, 751, and 770 amino acids, respectively (Wirak et al. (1991) Science 253:323). These isoforms of APP are generated by alternative splicing of primary transcripts of the APP gene, which is located on chromosome 21. It is known that APP isoforms are glycosylated transmembrane proteins (Goldgaber et al. (1987) Science 235: 877), and that two of the isoforms APP751 and APP770 have a protease inhibitor domain that is homologous to the Kunz type of serine protease inhibitors. The A β protein segment comprises approximately half of the transmembrane domain and approximately the first 28 amino acids of the extracellular domain of an APP isoform.

APP is a transmembrane protein which is highly expressed in all parts of the body, and which has several important biological functions. Proteolytic processing of APP in vivo is a normal physiological process. Carboxy-terminal truncated forms of APP695, APP751, and APP770 are present in brain and cerebrospinal fluid (Palmer et al. (1989) PNAS 86:6338; Weidemann et al. (1989) Cell 57:115) and result from cleavage of the APP at a constitutive cleavage site within the A β peptide domain of an APP isoform (Esch et al. (1990) Science 248:1122). There are probably two main metabolic pathways: one non-amyloid-forming and one amyloid-forming pathway. The non-amyloid-forming normal pathway starts by cleaving APP by the still unidentified enzyme α -secretase. This enzyme cleaves APP within the A β -fragment at amino acid no. 16 outside the cell wall (See fig. 1). A large (approximately 100 kD) soluble, N-terminal fragment, referred to as sAPP, containing a protease inhibitor domain in some isoforms, and a 9 kD membrane-bound, C-terminal fragment including most of the A β protein domain are generated. Studies have shown that sAPP, which is secreted from the cells, assists in training and in memory functions (Almkvist et al. (1997) Arch. Neurol. 54:641; Meziane et al. (1998) PNAS 95:12683).

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The second metabolic pathway resulting in generation of pathogenic A β protein apparently starts with aberrant proteolytic processing of APP, such that the normal cleavage at the constitutive site within the A β protein does not occur. Instead, cleavage occurs at two specific sites which flank the A β protein domain. One of these aberrant cleavage sites is in the transmembrane domain and the other aberrant cleavage site is located approximately at the N-terminus of the first 28 amino acids of the extra-cellular domain. The transmembrane cleavage is carried out by an enzyme called β -secretase. The other cleavage is performed by an enzyme called γ -secretase (Selkoe, D. J. (1994) *Annu Rev Neurosci* 17:489). Such aberrant proteolytic cleavage produces the A β protein polypeptide which is prone to forming dense amyloidogenic aggregates that are resistant to proteolytic degradation and removal. The resultant A β protein aggregates presumably are involved in the formation of the abundant amyloid plaques and cerebrovascular amyloid that are the neuropathological hallmarks of AD. However, the exact aberrant cleavage sites are not always precise; A β molecules isolated from the brain of a patient with AD show N- and C-terminal heterogeneity. Therefore, the aberrant cleavage pathway may also involve either sequence-specific proteolysis followed by exopeptidase activity (creating endoheterogeneity), or may not be sequence-specific.

In AD brains, the A β peptide forms virtually insoluble amyloid fibrils that accumulate into senile plaques. The A β fibrillization process is a complex multistep reaction. A group of distinct intermediary A β species of the fibrillization reaction, the protofibrils, were recently identified (D. M. Walsh, A. Lomakin, G. B. Benedek, M. M. Condron, D. B. Teplow, *J Biol Chem* **272**, 22364-22372 (1997), D. M. Walsh, et al., *J Biol Chem* **36**, 25945-25952 (1999), J. D. Harper, S. S. Wong, C. M. Lieber, P. T. Lansbury, *Biochemistry* **38**, 8972-8980 (1999)). Although little is known about the role of protofibrils in pathogenesis, recent findings indicate that protofibrils formed by another protein, α -synuclein, are involved in early onset Parkinson's disease. Pathogenic missense mutations in α -synuclein have been shown to accelerate protofibril formation (K. A. Conway, et al., *Proc Natl Acad Sci USA* **97**, 571-576 (2000)). Thus, protofibrils may have general importance as triggers of neurodegeneration.

It has long been presumed that A β only occurs in pathogenic conditions, and that a cell membrane damage is necessary in order to release the peptide. However, it has been shown that A β is also continuously produced in healthy individuals (Selkoe (1994) *supra*).

APP cleavage by the γ -secretase provides different forms of A β . The most common form comprises 40 amino acids (A β 40), but an A β comprising 42 amino acids (A β 42) has also been found in plaque and cerebrospinal fluid (CSF) (Scheuner et al. (1996) *Nature Med* 2:864). This longer form tends more to aggregate and it is believed that it is more pathogenic than A β 40.

Many patients get Alzheimer's disease spontaneously, but there are also several hereditary components involved. During the last years, there has been carried out an intensive mapping of genes involved in Alzheimer's disease. Disease-causing mutations in genes on chromosomes 1, 14, and 21, respectively, have been discovered, and these mutations might explain as much as 50 % of disease forms starting very early (<50 years)(St. George-Hyslop et al. (1987) *Science* 235:885; Sherrington et al. (1995) *Nature* 375:754).

The first gene associated with Alzheimer's disease was the gene encoding the amyloid precursor protein (APP) on chromosome 21. Different mutations of this gene result in unusual hereditary forms of the disease. US, A, 5,455,169 and Mullan et al. (1992), *Nature Genet.* 1:345. Several pathogenic mutations have been identified in the (APP) gene, all located close to the major APP processing sites, for review see (J. Hardy, *Trends Neurosci.* 20, 154-159 (1997), D. J. Selkoe, *Nature* 399, A23-A31 (1999)). These processing sites are either located adjacent to the boundaries of the A β domain in APP (the β - and γ -secretase sites) or within the A β sequence itself (α -secretase site). An increased production of the more amyloidogenic A β 42 peptide is seen with mutations positioned in the vicinity of the γ -secretase cleavage site.

The only known AD mutation close to the β -secretase site, the Swedish mutation (M. Mullan, et al., *Nature Genet* 1, 345-347 (1992)), discloses a double mutation (Lys670Asn/Met671Leu) of the APP gene in a large Swedish family, in which family the disease starts early and has a high penetrating power. The mutation produces a large increase of A β production, an elevation of both A β 42 and A β 40 in plasma from mutation carriers and in conditioned cell media (M. Citron, et al., *Proc Natl Acad Sci USA* 91, 11993-11997 (1994), J. A. Johnston, et al., *FEBS Lett* 354, 274-278 (1994), D. Scheuner, et al., *Nature Med* 2, 864-869 (1996)). It has been used as a model system for Alzheimer's disease in transgenic mice and transfected cells.

Other APP mutations have been described. All result in an early starting Alzheimer's disease having an autosomal dominant heredity pattern. Pathogenic mutations within the A β sequence (Fig. 3a), located close to the α -secretase site, result in a phenotype different from AD, with massive amyloid accumulation in cerebral blood vessel walls. Two mutations at codons 692 and 693, namely the Dutch (Glu693Gln) and the Flemish (Ala692Gly) mutations, have been reported (Levy et al. (1990) *Science* 248:1124; van Broeckhoven et al. (1990) *Science* 248:1120; Hendriks et al. (1992) *Nature Genet.* 1:218). These mutations have not been sufficiently characterized but patients having these mutations suffer from Alzheimer's disease comprising vascular symptoms. The vascular symptoms are caused by aggregation of A β in blood vessel walls (amyloid angiopathy). The Dutch mutation carriers (E693Q) are clinically characterized by the occurrence of intracerebral hemorrhages (E. Levy, et al., *Science* 248, 1124-1126 (1990)). Carriers of the Flemish mutation (A692G), frequently suffer from intracerebral hemorrhage, and individuals who survive develop a progressive dementia with features of AD (L. Hendriks, et al., *Nature Genet* 1, 218-221 (1992)). A third pathogenic intra-A β mutation was recently discovered in an Italian family (E693K), with clinical findings similar to the Dutch patients (F. Tagliavini, et al., *Alz Report* 2, S28 (1999)).

Different pathogenic mechanisms have been proposed for the Dutch and Flemish mutations. It has been observed that the Flemish mutation leads to increased A β levels while a reduced ratio of A β 42/40 was seen in media from cells transfected with the Dutch mutation (C. De Jonghe, et al., *Neurobiol Disease* 5, 281-286 (1998)). In addition, the Flemish mutation results in an increased A β /p3 ratio (Fig.3a), which was not observed for the Dutch mutation (C. Haass, A. Y. Hung, D. J. Selkoe, D. B. Teplow, *J Biol Chem* 269, 17741-17748 (1994), D. J. Watson, D. J. Selkoe, D. B. Teplow, *Biochem J* 340, 703-709 (1999)). Investigations of synthetic A β peptides have indicated that the Dutch mutation, but not the Flemish, accelerates the fibril formation compared to wild-type (wt) peptide (D. M. Walsh, A. Lomakin, G. B. Benedek, M. M. Condron, D. B. Teplow, *J Biol Chem* 272, 22364-22372 (1997)). Distinct A β fibrillization kinetics and effects on APP metabolism by these mutations may thus underly the differences in their clinical expression.

As reported by Kamino et al. (K. Kamino, et al., *Am J Hum Genet* 51, 998-1014 (1992)), another APP E693 mutation wherein Glu is substituted for Gly at APP E693, has previously

been characterized. It could not be unambiguously determined to be responsible for AD, though. This case originated from a family with similar clinical characteristics for AD and definitive AD was confirmed at autopsy. However, in this family the mutation could only be detected in one of two demented siblings.

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Several mutations at codon 717 of APP, in the vicinity of the cleavage point of the γ -secretase, have also been reported (Goate et al. (1991) Nature 349:704; Chartier-Harlin et al. (1991) Nature 353:844; Murrel et al. (1991) Science 254:97). These mutations specifically increase production of A β 42 which is more apt to aggregate.

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Due to the large costs and suffering that are associated with Alzheimer's disease, there is a need for methods for determining presence of hitherto unknown disease-associated mutations as well as knowledge about which mutations that causes familial Alzheimer's disease (AD). Likewise, there is a need for a method for screening compounds that could constitute a part of future pharmaceutical preparations for treating and perhaps curing Alzheimer's disease.

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Brief description of the invention

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The present invention relates to the identification of a pathogenic AD mutation at codon 693 (G1u693G1y), named the 'Arctic mutation', located within the A β peptide domain of the APP gene. Carriers of this mutation develop progressive dementia with clinical features typical of AD without symptoms of cerebrovascular disease. Said AD is distinctly characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said arctic mutation, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, and by accelerated formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) compared to protofibril formation of wild type A β peptides. Accordingly, the present invention provides an isolated nucleic acid characteristic of human amyloid precursor protein 770 including the nucleotides encoding codon 693, wherein the nucleic acid encodes glycine. Also provided is a method for diagnosing or predicting a predisposition to Alzheimer's disease and a polypeptide corresponding to the arctic mutation APP as well as antibodies generated against said polypeptide. The present invention also provides a transgenic non-human mammal comprising a gene encoding

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amyloid precursor protein mutated at codon 693 in such a way that this codon encodes glycine.

Detailed description of the invention

5 Several pathogenic Alzheimer's disease (AD) mutations have previously been described, all of which cause increased amyloid β -peptide ($A\beta$) levels. The present invention presents a pathogenic amyloid precursor protein (APP) mutation located within the $A\beta$ sequence at codon 693 (E693G), causing AD in a family from northern Sweden. Surprisingly, carriers of this "Arctic" mutation show decreased $A\beta$ 42 and $A\beta$ 40 levels in plasma. This finding is
10 corroborated *in vitro*, where the $A\beta$ 42 concentration was low in conditioned media from cells transfected with APP_{E693G}. Fibrillization studies demonstrate that $A\beta$ peptides with the Arctic mutation ($A\beta$ 1–40Arc) form protofibrils at a much higher rate and in larger quantities than wild-type (wt) $A\beta$. The unique finding of decreased $A\beta$ plasma levels in the Arctic AD family highlights the complexity of the disease and is likely to reflect a novel pathogenic
15 mechanism. The mechanism disclosed in the present invention involves a rapid $A\beta$ protofibril formation leading to accelerated build-up of insoluble $A\beta$ intra- and/or extracellularly.

The identified APP mutation (E693G) of the present invention was discovered in a family
20 from northern Sweden, named the "Arctic" family, which spans over four generations (Fig. 3b). Clinical information was available on eleven affected cases in three generations. An autosomal dominant pattern of inheritance was seen in the family with a mean age of onset at 57 ± 2.9 years (range 54 to 61 years). Clinical examination, neuropsychological testing, brain imaging (computed tomography or magnetic resonance imaging) and EEG were used
25 in evaluating patients according to DSM-IV criteria. Clinical history was typical for AD, with a slow insidious progression and decline in memory for recent events as the first presenting symptom. Signs of strokes or vascular lesions were not seen on brain imaging in seven investigated patients. The family was screened for mutations in exons 16 and 17 of the APP gene by single strand conformation polymorphism analysis (SSCP) (L. Forsell,
30 L. Lannfelt, *Neurosci Lett* **184**, 90-93 (1995)). An abnormal mobility pattern was observed in exon 17. Sequencing revealed an A→G nucleotide substitution, representing a glutamic acid to a glycine substitution at APP codon 693 (E693G), corresponding to position 22 in the $A\beta$ sequence. The mutation was further verified by restriction analysis, since it

destroyed a MbolI restriction site. The mutation was fully penetrant as no escapees were found. Two-point linkage analysis was performed between the mutation and affection status in the family with an age-dependent penetrance, giving a lod score of 3.66 at recombination fraction 0.00.

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- It has previously been shown that A β 42 is elevated in plasma from subjects with APP717 mutations as well as with presenilin (PS) 1 and PS 2 mutations. In carriers of the Swedish mutation (APP KM670/671NL) both A β 42 and A β 40 are elevated in plasma (D. Scheuner, et al., *Nature Med* 2, 864-869 (1996). M. Citron, et al., *Nature Med* 3, 67-72 (1997)). On the basis of these findings, it has been suggested that overproduction of A β 42 is a central event in the pathogenesis of AD. Inconclusive results on A β plasma levels in sporadic AD have been reported (D. Scheuner, et al., *Nature Med* 2, 864-869 (1996). A. Tamaoka, et al., *J Neurol Sci* 15, 65-68 (1996), T. Iwatsubo, *Neurobiol Aging* 19, 161-163 (1998), R. Mayeux, et al., *Ann Neurol* 46, 412-416 (1999)). Surprisingly, the present invention shows that A β 42 and A β 40 plasma levels are significantly decreased in carriers of the Arctic mutation. Low levels of A β are also observed in the youngest mutation carriers investigated, 20-30 years before the expected onset of the disease. The other three intra-A β mutations identified (Dutch, Flemish, and Italian) give rise to a clinical phenotype different from AD.
- APP and PS mutations studied in cell culture have given results later proven to reflect processes *in vivo*. Thus, the Arctic mutation was compared to the other intra-A β mutations in transiently transfected cells. Strikingly, all three mutations at codon 693 (Arctic, Dutch and Italian) lead to decreased A β 42 concentrations in conditioned media, whereas increased levels of both A β 42 and A β 40 in media was found for the Flemish APP692 mutation. It is intriguing that mutations located at the same codon of APP lead to such different phenotypes, while *in vitro* studies indicate a similar effect on A β concentration in conditioned media. It is evident that A β deposition is a central event in the pathological cascade. The answer lies within the A β sequence. It has been shown that the KLVFF motif at position 16-20 in the A β peptide is central in the fibrillization process (L. O. Tjernberg, et al., *J Biol Chem* 271, 8545-8548 (1996)).
33. C. Soto, M. S. Kindy, M. Baumann, B. Frangione, *Biochem Biophys Res Commun* 226, 672-680 (1996)). Mutations at position 21 and 22 in A β are located close to the KLVFF region and can therefore affect the conformation of the peptide and its fibrillization process.

In the present invention, the single amino acid substitution Glu to Gly at position 22 in the A β 1-40Arc molecule was found to cause a dramatic increase in rate and capacity to form protofibrils compared to the A β 1-40wt peptide. Thus, when A β 42Arc and A β 40Arc are formed in the brain it is likely that they are more prone to be retained by cellular systems
5 since the accelerated drive to form protofibrils enhances both A β bulk and insolubility.

Recent cell biological studies support the view that A β is generated intracellularly (for review see C. A. Wilson, R. W. Doms, V. M. Lee, *J Neuropath Experiment Neurol* 58, 787-794 (1999)). In addition, it has been reported that human neurons preferably accumulate
10 A β 42 over A β 40 (G. K. Gouras, et al., *Am J Pathol* 156, 15-20 (2000)). This finding is in line with studies showing that C-terminally extended peptides have a much higher tendency to form amyloid fibrils (J. D. Harper, P. T. J. Lansbury, *Ann Review Biochem* 66, 385-407 (1997)). This offers an explanation to the decreased A β 42 concentration in media from cells transfected with mutations at APP codon 693.

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The unique finding of decreased A β plasma levels in the Arctic AD family highlights the complexity of the disease and is likely to reflect a novel pathogenic mechanism. The present invention proposes that this mechanism involves a rapid A β protofibril formation leading to accelerated build-up of insoluble A β intra- and/or extracellularly. In this context it
20 is interesting to note that the vast majority of sporadic AD cases do not show increased A β plasma levels. Thus, factors promoting protofibril formation should be considered in the pathogenesis of sporadic AD. Increased protofibril formation is probably also operating in these more common forms of the disease. Indeed, the findings of the present invention open new avenues for possible therapeutic intervention using drugs targeted at preventing
25 protofibril formation (K. A. Conway, et al., *Proc Natl Acad Sci USA* 97, 571-576 (2000)). The Arctic form of AD may be an example of a rare disease variant that sheds light on molecular mechanisms underlying more common forms of the disorder. The present findings suggest a previously not described pathogenic mechanism for AD: an intra-A β mutation leading to rapid A β protofibril formation and decreased plasma A β levels, they
30 constitute an important tool for achieving increased understanding of how Alzheimer's disease starts.

Accordingly, the present invention provides an isolated nucleic acid characteristic of human amyloid precursor protein (APP) including the nucleotides encoding codon 693 of human

APP, wherein the nucleic acid encodes glycine and/ or a subfragment thereof including the nucleotides encoding codon 693.

The invention also provides an isolated nucleic acid complementary to the nucleic acid of the invention. The isolated nucleic acid can be labeled with a detectable moiety. The isolated nucleotide can encode, for example, AD and the entire human APP770. The invention also provides polypeptides encoded by these nucleic acids.

Further, the invention provides an antibody specifically reactive with the polypeptides of the invention. Thus, antibodies which react with the unique epitope created by glycine at codon 693 are provided.

Vectors comprising the nucleic acids of the invention are also provided. These vectors can be placed in a host capable of expressing the characteristic portion of human APP.

The invention also provides a method of diagnosing or predicting a predisposition to AD. The method comprises detecting in a sample from a subject the presence of a mutation in a human APP in a position corresponding to codon 693 of human amyloid precursor protein 770 (APP770) or a fraction thereof, the presence of the mutation indicating the presence of a predisposition to AD. As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides of the mutation and detecting the presence of the mutation in the amplified product. Further, the detecting step can comprise selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. The mutation can also be detected by cleaving with restriction enzyme MbolI as the mutation changes a MbolI site (The site is changed from the MbolI recognition sequence' (AAG, native APP form) to ~GGAGA Arctic APP form) and the MbolI site is destroyed).

Pathogenic APP mutations have been shown to affect APP processing, as reflected in an increase of either total A β or A β 42 in the plasma of affected family members. The Arctic mutation of the present invention is located in a region different from other AD-causing mutations. The present invention shows that the mutation manifests itself by affecting A β

plasma levels. Plasma from nine mutation carriers, of which four were symptomatic, and eleven non-carriers in the family was analysed. The A β 42 plasma concentration was 11.7 ± 3.9 fmol/ml and 16.0 ± 5.6 fmol/ml in mutation carriers and non-carriers, respectively (Fig. 4), representing a 27% reduction of A β 42 in the mutation carriers ($p=0.04$). The A β 40 plasma concentration was 105 ± 22 fmol/ml and 141 ± 34 fmol/ml in mutation carriers and non-carriers, respectively (Fig. 2), representing a 26% reduction of A β 40 in the mutation carriers ($p=0.01$). In conclusion, concentrations of both A β 42 and A β 40 were unexpectedly and significantly reduced in individuals carrying the Arctic mutation. Therefore, the present invention relates to a AD wherein the A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said arctic mutation are decreased at least between 10-30%, such as at least 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.

Said AD is further characterised by accelerated formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) compared to protofibril formation of wild type A β peptides. The formation is accelerated at least 2-10 times compared to protofibril formation of wild type A β peptides, such as at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times or at least 10 times.

The invention also provides a transgenic non-human animal containing, in a germ or somatic cell, the mutated nucleic acid of the invention, wherein the animal expresses a human APP or a fragment thereof which encodes glycine instead of glutamic acid at codon 693. Preferably, the animal expresses neuropathological characteristics of AD. Preferably, the mutated APP is expressed in cells which normally express the naturally-occurring endogenous APP gene (if present). Typically, the non-human animal is a mouse. Such transgenes typically comprises an Arctic mutation APP expression cassette, wherein a linked promoter and, preferably, an enhancer drive expression of structural sequences encoding a heterologous APP polypeptide comprising the Arctic mutation.

The invention also provides transgenes comprising a gene encoding an Arctic mutation APP, said gene operatively linked to a transcription regulatory sequence functional in the host transgenic animal (e.g. a neural-specific promoter). Such genes are typically integrated into a host chromosome location by non-homologous integration. The transgenes may further comprise a selectable marker, such as a neo or gpt gene

operatively linked to a constitutive promoter, such as a phosphoglycerate kinase (pgk) promoter or HSV tk gene promoter linked to an enhancer (e.g., SV40 enhancer).

5 The invention further provides non-human transgenic animals, typically non-human mammals such as mice or rats, which animals harbor at least one copy of a transgene or targeting construct of the invention, either homologously or nonhomologously integrated into an endogenous chromosomal location so as to encode an Arctic mutation APP polypeptide. Such transgenic animals are usually produced by introducing the transgene or targeting construct into a fertilized egg or embryonic stem (ES) cell, typically by
10 microinjection, electroporation, lipofection, or biolistics. The transgenic animals express the Arctic mutation APP gene of the transgene (or homologously recombined targeting construct), typically in brain tissue. Such animals are suitable for use in a variety of disease models and drug screening uses, as well as other applications.

15 The invention also provides non-human animals and cells which harbor at least one integrated targeting construct that functionally disrupts an endogenous APP gene locus, typically by deleting or mutating a genetic element (e.g., exon sequence, splicing signal, promoter, enhancer) that is required for efficient functional expression of a complete gene product.

20 The invention also provides transgenic non-human animals, such as a non-primate mammal, that have at least one inactivated endogenous APP allele, and preferably are homozygous for inactivated APP alleles, and which are substantially incapable of directing the efficient expression of endogenous (i.e., wild-type) APP. For example, in a preferred
25 embodiment, a transgenic mouse is homozygous for inactivated endogenous APP alleles and is substantially incapable of producing murine APP encoded by a endogenous (i.e., naturally-occurring) APP gene. Such a transgenic mouse, having inactivated endogenous APP genes, is a preferred host recipient for a transgene encoding a heterologous APP polypeptide, preferably a human Arctic mutation APP polypeptide. For example, human
30 APP comprising the Arctic mutation may be encoded and expressed from a heterologous transgene(s) in such transgenic mice. Such heterologous transgenes may be integrated in a non-homologous location in a chromosome of the non-human animal, or may be integrated by homologous recombination or gene conversion into a non-human APP gene locus, thereby effecting simultaneous knockout of the endogenous APP gene (or segment
35 thereof) and replacement with the human APP gene (or segment thereof).

The invention also provides a host containing the nucleic acid of claim 1, which host expresses a human APP or a fragment thereof, which encodes glycine at codon 693. Preferably, the host is an immortalized cell line. The invention also provides a method for screening for an agent capable of treating AD. The method comprises contacting these transgenic animals or host cell lines with the agent and monitor the expression, processing or deposition of APP or fragments thereof.

As used herein, "isolated" means free of at least some of the contaminants associated with the nucleic acid or polypeptides occurring in a natural environment.

As used herein, "nucleic acid characteristic of human amyloid precursor protein" refers to a nucleic acid which has sufficient nucleotides surrounding the codon at position 693 to distinguish the nucleic acid from nucleic acids encoding non-related proteins. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in codon 693, the length of the nucleic acid will be smaller, but must be long enough to prevent hybridization to background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially between 10 and 40 nucleotides in length.

Likewise, polypeptides encoded by the nucleic acids of the invention can be variable depending on the desired function of the polypeptide. While smaller fragment can work, generally to be useful, e.g. to be immunogenic, the polypeptide must be of at least 8 amino acids and not more than 10,000 amino acids. Below is an example of a small immunogenic peptide according to the present invention comprising codons 689 - 697:

Val - Phe - Phe - Ala - Gly - Asp - Val - Gly - Ser

The amino and carboxy ends of this core sequence can include any number of additional amino acids from the APP sequence. Thus, "fragment" can be a truncated APP isoform or a modified APP isoform (as by amino acid substitutions, deletions or additions). This definition recognizes that APP is a single gene that undergoes alternative splicing to generate several isoforms that are designated by the total number of amino acids in each.

Thus treatment includes various alternatively spliced exons resulting in isoforms of 770, 751, 714, 695, 563 and 365 amino acids.

As used here, "codon 693" refers to the codon (i.e. the tri-nucleotide sequence) that
5 encode the 693rd amino acid position in APP770. For example, a 570 residue-long
fragment that is produced by truncating APP770 by removing the 100 N-terminal amino
acids has its 493rd amino acid position corresponding to codon 693. In fact, as used
herein, codon 693 refers to the codon that encodes the 674th amino acid residue of
APP751 and the 618th amino acid residue of APP695. In addition codon 693 refers to the
10 complementary sequence on the antisense strand.

As used herein, the term "mutant" refers specifically to a mutation at codon 693 (as
referenced by the amino acid sequence in APP770) of the APP gene, such that codon 693
encodes glycine.

15

It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions
may be incorporated into the polynucleotides of the invention. However, such nucleotide
substitutions, deletions, and additions should not substantially disrupt the ability of the
polynucleotide to hybridize to one of the polynucleotide sequences under hybridizing
20 conditions that are sufficiently stringent to result in specific hybridization.

"Specific hybridization" is defined herein as the formation of hybrids between a probe
nucleic acid (e.g. a nucleic acid which may include substitutions, deletions, and/or
additions) and a specific target nucleic acid (e.g. a nucleic acid having the sequence),
25 wherein the probe preferentially hybridizes to the specific target such that, for example, a
band corresponding to a variant APP allele or restriction fragment thereof can be identified
on a Southern blot, whereas a corresponding wildtype APP allele (i.e. one that encodes
glutamic acid at codon 693) is not identified or can be discriminated from a variant APP
allele on the basis of signal intensity. Hybridization probes capable of specific hybridization
30 to detect a single-base mismatch may be designed according to methods known in the art
and described in Maniatis et al. (1989) *Molecular Cloning: A laboratory Manual*, 2nd Ed.,
Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. USA; Berger and Kimmel (1987)
"Guide to Molecular Cloning Techniques", *Methods in Enzymology*, Vol. 152, Academic
Press Inc., San Diego, California, USA; Gibbs et al. (1990) *Nucl. Acids Res.* 17:2437;

Kwork et al. (1990) Nucl. Acids Res. 18:999; and Miyada et al. (1987) Methods Enzymol. 154:94.

5 The vectors for expressing the polypeptides of the invention require that the nucleic acid be "operatively linked." A nucleic acid is operatively linked when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operatively linked to a coding sequence if it affects the transcription of the sequences. Operatively linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein-coding regions, contiguous and in reading-frame.

10

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological material such as bacteria, plants, fungi or animal (particularly mammalian) cells or tissues. Agents are evaluated for potential biological activity by inclusion in screening assays described herein
15 below.

20

As used herein, the terms "label" or "labeled" refer to labels commonly within the art of bioassays. Examples of such labels are groups comprising a radioactive nuclide, as well as enzymatic and fluorescent markers.

Accordingly, genetic alterations in the APP gene resulting in altered degradative properties are very important in the application of the invention. There are several methodologies available from recombinant DNA technology which may be used for detecting and identifying a genetic mutation responsible for AD. These include, for example, direct
25 probing, polymerase chain reaction (PCR) methodology, restriction fragment length polymorphism (RFLP) analysis and single strand conformational analysis (SSCA). However, any other known methods or later discovered methods can likewise be used to detect the mutation. Once the location of the 693 mutation is known and associated with AD, the methods to detect the mutation are standard in the art. One example of a suitable
30 technique is disclosed in EP 0 648 280. The sequence of various nucleotide probes can be determined from the sequence of APP, especially the sequences surrounding codon 693. The nucleic acid sequence of APP is set forth in Yoshikai et al. (1990) Gene 87:257.

The term "cognate" as used herein refers to a gene sequence that is evolutionarily and
35 functionally related between species. For example but not limitation, in the human genome,

the human immunoglobulin heavy chain gene locus is the cognate gene to the mouse immunoglobulin heavy chain gene locus, since the sequences and structures of these two genes indicate that they are highly homologous and both genes encode a protein which functions to bind antigens specifically.

5

As used herein, the term "xenogenic" is defined in relation to a recipient mammalian host cell or non-human animal and means that an amino acid sequence or polynucleotide sequence is not encoded by or present in, respectively, the naturally occurring genome of the recipient mammalian host cell or non-human animal. Xenogenic DNA sequences are foreign DNA sequences; for example, a human APP gene is xenogenic with respect to murine ES cells; also, for illustration, a human cystic fibrosis-associated CFTR allele is xenogenic with respect to a human cell line that is homozygous for wild-type (normal) CFTR alleles. Thus, a cloned murine nucleic acid sequence that has been mutated (e.g., by site directed mutagenesis) is xenogenic with respect to the murine genome from which the sequence was originally derived, if the mutated sequence does not naturally occur in the murine genome.

As used herein, a "heterologous gene" or "heterologous polynucleotide sequence" is defined in relation to the transgenic non-human organism producing such a gene product. A heterologous polypeptide, also referred to as a xenogeneic polypeptide, is defined as a polypeptide having an amino acid sequence or an encoding DNA sequence corresponding to that of a cognate gene found in an organism not consisting of the transgenic non-human animal. Thus, a transgenic mouse harboring a human APP gene can be described as harboring a heterologous APP gene. A transgene containing various gene segments encoding a heterologous protein sequence may be readily identified, e.g. by hybridization or DNA sequencing, as being from a species of organism other than the transgenic animal. For example, expression of human APP amino acid sequences may be detected in the transgenic non-human animals of the invention with antibodies specific for human APP epitopes encoded by human APP gene segments. A cognate heterologous gene refers to a corresponding gene from another species; thus, if murine APP is the reference, human APP is a cognate heterologous gene (as is porcine, ovine, or rat APP, along with APP genes from other species). A mutated endogenous gene sequence can be referred to as a heterologous gene; for example, a transgene encoding a murine APP comprising a Arctic mutation (which is not known in naturally-occurring murine genomes) is a heterologous transgene with respect to murine and non-murine species.

As used herein, the term "targeting construct" refers to a polynucleotide which comprises: (1) at least one homology region having a sequence that is substantially identical to or substantially complementary to a sequence present in a host cell endogenous gene locus, and (2) a targeting region which becomes integrated into an host cell endogenous gene locus by homologous recombination between a targeting construct homology region and said endogenous gene locus sequence. If the targeting construct is a "hit-and-run" or "in-and-out" type construct (Valancius and Smithies (1991) Mol. Cell. Biol. 11: 1402; Donehower et al. (1992) Nature 356: 215; (1991) J. NIH Res. 3: 59; Hasty et al. (1991) Nature 350: 243, which are incorporated herein by reference), the targeting region is only transiently incorporated into the endogenous gene locus and is eliminated from the host genome by selection. A targeting region may comprise a sequence that is substantially homologous to an endogenous gene sequence and/or may comprise a non-homologous sequence, such as a selectable marker (e.g., neo, tk, gpt). The term "targeting construct" does not necessarily indicate that the polynucleotide comprises a gene which becomes integrated into the host genome, nor does it necessarily indicate that the polynucleotide comprises a complete structural gene sequence. As used in the art, the term "targeting construct" is synonymous with the term "targeting transgene" as used herein.

Generally, the nomenclature used hereafter and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, cell culture, and transgene incorporation (e.g., electroporation, microinjection, lipofection). Generally enzymatic reactions, oligonucleotide synthesis, and purification steps are performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references which are provided throughout this document.

The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

Chimeric targeted mice are derived according to Hogan, et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory (1988) and

Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed., IRL Press, Washington, D.C., (1987) which are incorporated herein by reference.

Embryonic stem cells are manipulated according to published procedures

- 5 (Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed., IRL Press, Washington, D.C. (1987); Zijlstra et al., Nature 342:435-438 (1989); and Schwartzberg et al., Science 246:799-803 (1989), each of which is incorporated herein by reference).

- 10 Oligonucleotides can be synthesized on an Applied Bio Systems oligonucleotide synthesizer according to specifications provided by the manufacturer.

- In general, the invention encompasses methods and polynucleotide constructs which are employed for generating non-human transgenic animals expressing an APP polypeptide comprising the Arctic mutation. In some embodiments, the non-human transgenic animals expressing an Arctic mutation APP also have the endogenous APP gene locus functionally disrupted. Advantageously, the Arctic mutation results in enhanced production of A β , with animals or cells generally producing transgeneencoded Arctic mutation A β at a significantly higher level than normal A β . It is believed that the substitution of glu693 for gly693 is of particular importance in the preferential expression of the Arctic A β as compared to the normal (wild-type) A β .
- 15
- 20

- Newly identified secreted fragments comprise the amino-terminal portion of AP which remains after cleavage and will be referred to hereinafter as Arctic Ap. Arctic A β is believed to be the product of an alternative secretory processing pathway for A β , which pathway is present even in normal (non-diseased) cells. It is further believed, however, that the alternate secretory pathway may be responsible for an essential event in the production of A β in diseased cells in patients, and that abnormal production of A β may be involved in diseases related to A β plaque, particularly Alzheimer's disease and Down's syndrome.
- 25

- 30 Particularly preferred animal models for P-secretase cleavage of A β are transgenic animals which express the Arctic mutation of the A β gene, as described above. It has been found that such transgenic animals, particularly transgenic mice, produce high quantities of Arctic AP which may be detected according to the methods of the present invention. In particular, it

has been found that Arctic mutation results in quantities of Arctic A β which will usually be at least two-fold higher than wild type human A β expressed in animals. Usually, production will be significantly higher, typically being at least two-fold higher. With such elevated levels of A β production, monitoring P-secretase activity under different conditions is greatly facilitated. In particular, screening for drugs and other therapies for inhibiting P-secretase activity (and thus inhibiting Arctic A β production) are greatly simplified in animals models expressing the Arctic mutation of human APP.

Agents are administered to test animals, such as test mice, which are transgenic and which express the Arctic mutation of human APP. Particular techniques for producing transgenic mice which express the Arctic form of APP are described hereinafter. It will be appreciated that the preparation of other transgenic animals expressing the Arctic human APP may easily be accomplished, including rats, hamsters, guinea pigs, rabbits, and the like. The effect of test compounds on Arctic APP production in test animals may be measured in various specimens from the test animals.

The effect of test agents on Arctic A β production in test animals may be measured in various specimens from the test animals. In all cases, it will be necessary to obtain a control value which is characteristic of the level of A β production in the test animal in the absence of test compound(s). In cases where the animal is sacrificed, it will be necessary to base such control values on an average or a typical value from other test animals which have been transgenically modified to express the Arctic mutant of human aAPP but which have not received the administration of any test compounds or any other substances expected to affect the level of production of A β . Once such control level is determined, test compounds can be administered to additional test animals, where deviation from the average control value indicates that the test compound had an effect on the β -secretase activity in the animal. Test substances which are considered positive, i.e., likely to be beneficial in the treatment of Alzheimer's disease or other β -amyloid-related conditions, will be those which are able to reduce the level of A β production, preferably by at least 20%, more preferably by at least 50%, and most preferably by at least 80%.

The test agents can be any molecule, compound, or other substance which can be added to the cell culture or administered to the test animal without substantially interfering with cell or animal viability. Suitable test agents may be small molecules, biological polymers, such as polypeptides, polysaccharides, polynucleotides, and the like. The test compounds

will typically be administered to transgenic animals at a dosage of from 1 ng/kg to 10 mg/kg, usually from 10 pg/kg to 1 mg/kg.

- Test compounds which are able to inhibit secretion or animal production of Arctic AP are
- 5 considered as candidates for further determinations of the ability to block diamyloid production in animals and humans. Inhibition of secretion or production indicates that cleavage of APP at the amino-terminus has likely been at least partly blocked, reducing the amount of a processing intermediate available for conversion to A β .
- 10 The present invention further comprises pharmaceutical compositions incorporating a compound selected by the above-described method and said compound being included in a pharmaceutically acceptable carrier. Such pharmaceutical compositions should contain a therapeutic or prophylactic amount of at least one compound identified by the method of the present invention. The pharmaceutically acceptable carrier can be any compatible,
- 15 non-toxic substance suitable to deliver the compounds to an intended host. Sterile water, alcohol, fats, waxes, and inert solids may be used as the carrier. Pharmaceutically acceptable adjuvants, buffering agents, dispersing agents, and the like may also be incorporated into the pharmaceutical compositions. Preparation of pharmaceutical conditions incorporating active agents is well described in the medical and scientific
- 20 literature. See, for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 16th Ed., 1982, the disclosure of which is incorporated herein by reference.

The pharmaceutical compositions just described are suitable for systemic administration to

25 the host, including both parenteral, topical, and oral administration. The pharmaceutical compositions may be administered parenterally, i.e. subcutaneously, intramuscularly, or intravenously. Thus, the present invention provides compositions for administration to a host, where the compositions comprise a pharmaceutically acceptable solution of the identified compound in an acceptable carrier; as described above.

30

Transgenes Encoding Heterologous Arctic Mutation APP Protein

- In a preferred embodiment of the invention, a transgene encoding a heterologous APP protein comprising the Arctic mutation (glu693gly) is transferred into a fertilized embryo or
- 35 an ES cell to produce a transgenic non-human animal that expresses APP polypeptide(s)

comprising the Arctic mutation. A transgene encoding a heterologous Arctic mutation APP protein comprises structural sequences encoding a heterologous Arctic mutation APP protein, and generally also comprises linked regulatory elements that drive expression of the heterologous Arctic mutation APP protein in the non-human host. However,

5 endogenous regulatory elements in the genome of the non-human host may be exploited by integrating the transgene sequences into a chromosomal location containing functional endogenous regulatory elements which are suitable for the expression of the heterologous structural sequences. Such targeted integration is usually performed by homologous gene targeting as described supra, wherein the heterologous transgene would comprise at least

10 one homology clamp.

When a heterologous transgene relies on its own regulatory elements, suitable transcription elements and polyadenylation sequences) are included. At least one promoter is linked upstream of the first structural sequence in an orientation to drive transcription of the

15 heterologous structural sequences. Sometimes the promoter from the naturally-occurring heterologous gene is used (e.g., a human APP promoter is used to drive expression of a human Arctic mutation APP transgene). Alternatively, the promoter from the endogenous cognate APP gene may be used (e.g., the murine APP promoter is used to drive expression of a human Arctic mutation APP transgene). Alternatively, a transcriptional

20 regulatory element heterogeneous with respect to both the transgene encoding sequences and the non-human host animal can be used (e.g., a rat promoter and/or enhancer operably linked to a nucleotide sequence encoding human Arctic mutation APP, wherein the transgene is introduced into mice).

25 In some embodiments, it is preferable that the transgene sequences encoding the Arctic mutation APP polypeptide are under the transcriptional control of promoters and/or enhancers (and/or silencers) which are not operably linked in naturally occurring APP genes (i.e., non-APP promoters and/or enhancers). For example, some embodiments will employ transcriptional regulatory sequences which confer high level expression and/or in a

30 cell type-specific expression pattern (e.g., a neuron-specific promoter). The rat neural-specific enolase (NSE) promoter (ForssPetter (1990) Neuron 5; 187) is a preferred transcriptional regulatory element for operable linkage to a nucleotide sequence encoding an Arctic mutation APP polypeptide. Other promoters and/or enhancers which confer efficient expression to the transgene-encoded APP sequence in brain tissue generally are

35 preferred.

Various promoters having different strengths (e.g., pgk, tk, dhfr) may be substituted in the discretion of the practitioner, however it is essential that the promoter function in the non-human host and it is desirable in some embodiments that the promoter drive
5 expression in a developmental pattern or cell type-specific pattern (and at expression levels) similar to a naturally-occurring APP gene in a parallel host animal lacking the transgene.

A heterologous transgene generally encodes at least one full-length APP isoform (e.g., a
10 695aa isoform). The heterologous transgene may comprise a polynucleotide spanning the entire genomic APP gene or portion thereof, may comprise a minigene, may comprise a single contiguous coding segment (e.g., cDNA), or may comprise a combination thereof. Frequently, the transgene encodes a human APP polypeptide sequence comprising the Arctic mutation, however transgenes encoding non-human APP polypeptides comprising
15 the Arctic mutation may also be used. Generally, the transgene will encode a full-length naturally-occurring APP isoform (e.g., APP695, APP75 1, or APP770) further comprising the Arctic mutation.

The transgenes encoding APP polypeptides comprising the Arctic mutation will frequently
20 will also comprise one or more linked selectable marker (infra).

Transgenes encoding heterologous APP polypeptides comprising the Arctic mutation molecules may be transferred into the non-human host genome in several ways. A heterologous transgene may be targeted to a specific predetermined chromosomal location
25 by homologous targeting, as described supra for gene targeting. Heterologous transgenes may be transferred into a host genome in pieces, by sequential homologous targeting, to reconstitute a complete heterologous gene in an endogenous host chromosomal location. In contradistinction, a heterologous transgene may be randomly integrated separately from or without using a APP gene targeting construct. A heterologous transgene may be
30 co-transferred with an APP gene targeting construct and, if desired, selected for with a separate, distinguishable selectable marker and/or screened with PCR or Southern blot analysis of selected cells. Alternatively, a heterologous transgene may be introduced into ES cells prior to or subsequent to introduction of a APP gene targeting construct and selection therefor. Most conveniently, a heterologous transgene is introduced into the
35 germline of a non-human animal by non-homologous transgene integration via pronuclear

injection, and resultant transgenic lines are bred into a homozygous knockout background having functionally disrupted cognate endogenous APP gene. Homozygous knockout mice can also be bred and the heterologous Arctic mutation APP transgene introduced into embryos of knockout mice directly by standard pronuclear injection or other means known in the art.

Gene Targeting

In some embodiments, the endogenous non-human APP alleles are functionally disrupted so that expression of endogenously encoded APP is suppressed or eliminated, so as to not interfere or contaminate transgene-encoded APP comprising the Arctic mutation. In one variation, an endogenous APP allele is converted to comprise the Arctic mutation by homologous gene targeting.

Gene targeting, which is a method of using homologous recombination to modify a mammalian genome, can be used to introduce changes into cultured cells. By targeting a gene of interest in embryonic stem (ES) cells, these changes can be introduced into the germlines of laboratory animals to study the effects of the modifications on whole organisms, among other uses. The gene targeting procedure is accomplished by introducing into tissue culture cells a DNA targeting construct that has a segment homologous to a target locus and which also comprises an intended sequence modification (e.g., insertion, deletion, point mutation). The treated cells are then screened for accurate targeting to identify and isolate those which have been properly targeted. A common scheme to disrupt gene function by gene targeting in ES cells is to construct a targeting construct which is designed to undergo a homologous recombination with its chromosomal counterpart in the ES cell genome. The targeting constructs are typically arranged so that they insert additional sequences, such as a positive selection marker, into coding elements of the target gene, thereby functionally disrupting it. Targeting constructs usually are insertion-type or replacement-type constructs (Hasty et al. (1991) *Mol Cell. Biol.* 11: 4509).

Targeting of the Endogenous APP Gene

The invention encompasses methods to produce non-human animals (e.g., nonprimate mammals) that have the endogenous APP gene inactivated by gene targeting with a homologous recombination targeting construct. Typically, a non-human APP gene

sequence is used as a basis for producing PCR primers that flank a region that will be used as a homology clamp in a targeting construct. The PCR primers are then used to amplify, by high fidelity PCR amplification (Mattila et al. (1991) *Nucleic Acids Res.* 19: 4967; Eckert, K. A. and Kunkel, T. A. (1991) *PCR Methods and Applications* 1: 17; U.S. Pat. No. 4,683,202, which are incorporated herein by reference), a genomic sequence from a genomic clone library or from a preparation of genomic DNA, preferably from the strain of non-human animal that is to be targeted with the targeting construct. The amplified DNA is then used as a homology clamp and/or targeting region. Thus, homology clamps for targeting a non-human APP gene may be readily produced on the basis of nucleotide sequence information available in the art and/or by routine cloning. General principles regarding the construction of targeting constructs and selection methods are reviewed in Bradley et al. (1992) *Bio/Technology* 10: 534, incorporated herein by reference.

Endogenous non-human APP genes may be functionally disrupted and, optionally, may be replaced by transgenes encoding APP comprising the Arctic mutation.

Targeting constructs can be transferred into pluripotent stem cells, such as murine embryonal stem cells, wherein the targeting constructs homologously recombine with a portion of an endogenous APP gene locus and create mutation(s) (i. e., insertions, deletions, rearrangements, sequence replacements, and/or point mutations) which prevent the functional expression of the endogenous APP gene.

A preferred method of the invention is to delete, by targeted homologous recombination, essential structural elements of the endogenous APP gene. For example, a targeting construct can homologously recombine with an endogenous APP gene and delete a portion spanning substantially all of one or more of the exons to create an exon-depleted allele, typically by inserting a replacement region lacking the corresponding exon(s). Transgenic animals homozygous for the exon-depleted allele (e.g., by breeding of heterozygotes to each other) produce cells which are essentially incapable of expressing a functional endogenous APP polypeptide (preferably incapable of expressing any of the naturally-occurring isoforms). Similarly, homologous gene targeting can be used, if desired, to functionally disrupt an APP gene by deleting only a portion of an exon.

Targeting constructs can also be used to delete essential regulatory elements of an endogenous APP gene, such as promoters, enhancers, splice sites, polyadenylation sites,

and other regulatory sequences, including cis-acting sequences that occur upstream or downstream of the APP structural gene but which participate in endogenous APP gene expression. Deletion of regulatory elements is typically accomplished by inserting, by homologous double-crossover recombination, a replacement region lacking the
5 corresponding regulatory element(s).

A alternative preferred method of the invention is to interrupt essential structural and/or regulatory elements of an endogenous APP gene by targeted insertion of a polynucleotide sequence, and thereby functionally disrupt the endogenous APP gene. For example, a
10 targeting construct can homologously recombine with an endogenous APP gene and insert a non-homologous sequence, such as a neo expression cassette, into a structural element (e.g., an exon) and/or regulatory element (e.g., enhancer, promoter, splice site, polyadenylation site) to yield a targeted APP allele having an insertional interruption. The
15 inserted sequence can range in size from about 1 nucleotide (e.g., to produce a frameshift in an exon sequence) to several kilobases or more, as limited by efficiency of homologous gene targeting with targeting constructs having a long non-homologous replacement region.

Targeting constructs of the invention can also be employed to replace a portion of an
20 endogenous APP gene with an exogenous sequence (i.e., a portion of a targeting transgene); for example, the first exon of an APP gene may be replaced with a substantially identical portion that contains a nonsense or missense mutation.

Inactivation of an endogenous mouse APP locus is achieved by targeted disruption of the
25 appropriate gene by homologous recombination in mouse embryonic stem cells. For inactivation, any targeting construct that produces a genetic alteration in the target APP gene locus resulting in the prevention of effective expression of a functional gene product of that locus may be employed. If only regulatory elements are targeted, some low-level expression of the targeted gene may occur (i.e., the targeted allele is "leaky"), however the
30 level of expression may be sufficiently low that the leaky targeted allele is functionally disrupted.

Generation of Null APP Alleles and Knockout Mice

In one embodiment of the invention, an endogenous APP gene in a non-human host is functionally disrupted by homologous recombination with a targeting construct that does not comprise a cognate heterologous APP gene segment comprising the Arctic mutation. In this embodiment, a portion of the targeting construct integrates into an essential
5 structural or regulatory element of the endogenous APP gene locus, thereby functionally disrupting it to generate a null allele. Typically, null alleles are produced by integrating a non-homologous sequence encoding a selectable marker (e.g., a neo gene expression cassette) into an essential structural and/or regulatory sequence of an APP gene by homologous recombination of the targeting construct homology clamps with endogenous
10 APP gene sequences, although other strategies (see, *infra*) may be employed.

Most usually, a targeting construct is transferred by electroporation or microinjection into a totipotent embryonal stem (ES) cell line, such as the murine AB-1 or CCE lines. The targeting construct homologously recombines with endogenous sequences in or flanking
15 an APP gene locus and functionally disrupts at least one allele of the APP gene. Typically, homologous recombination of the targeting construct with endogenous APP locus sequences results in integration of a nonhomologous sequence encoding, and expressing a selectable marker, such as neo, usually in the form of a positive selection cassette (*infra*). The functionally disrupted allele is termed an APP null allele. ES cells having at least one
20 APP null allele are selected for by propagating the cells in a medium that permits the preferential propagation of cells expressing the selectable marker. Selected ES cells are examined by PCR analysis and/or Southern blot analysis to verify the presence of a correctly targeted APP allele. Breeding of non-human animals which are heterozygous for a null allele may be performed to produce non-human animals homozygous for said null
25 allele, so-called "knockout" animals (Donehower et al. (1992) *Nature* 256: 215; *Science* 256: 1392, incorporated herein by reference). Alternatively, ES cells homozygous for a null allele having an integrated selectable marker can be produced in culture by selection in a medium containing high levels of the selection agent (e.g., G418 or hygromycin). Heterozygosity and/or homozygosity for a correctly targeted null allele can be verified with
30 PCR analysis and/or Southern blot analysis of DNA isolated from an aliquot of a selected ES cell clone and/or from tail biopsies.

If desired, a transgene encoding a heterologous APP polypeptide comprising the Arctic mutation can be transferred into a non-human host having an APP null allele, preferably
35 into a non-human ES cell that is homozygous for the APP null allele. It is generally

advantageous that the transgene comprises a promoter and enhancer which drive expression of structural sequences encoding a functional heterologous Arctic mutation APP gene product. Thus, for example and not limitation, a knockout mouse homozygous for null alleles at the APP locus is preferably a host for a transgene which encodes and expresses a functional human APP protein comprising the Arctic mutation. Arctic mutation APP transgenes comprise heterologous APP structural sequences encoding APP polypeptides comprising the Arctic mutation, either in the form of exons having splice junction sequences, as a contiguous coding segment (e.g., a cDNA), or as a combination of these. Most usually, Arctic mutation APP transgenes encode full-length APP polypeptides, although transgenes can encode truncated APP isoforms, chimeric APP polypeptides (e.g., part human/part mouse), and/or amino-substituted APP variants (i.e., mutems) further comprising the Arctic mutation. Typically, transgenes also comprise regulatory elements, such as a promoter and, for optimal expression, an enhancer.

Homologous APP Gene Replacement

In an alternative variation of the invention, an endogenous APP gene in a nonhuman host is functionally disrupted by homologous integration of a cognate heterologous APP gene comprising the Arctic mutation, such that the cognate heterologous APP gene substantially replaces the endogenous APP gene, at least spanning the amino acid 595-596 positions according to the Kang et al. (1987) optic numbering convention, and preferably completely replaces the coding sequences of the endogenous APP gene. Preferably, the heterologous Arctic mutation APP gene is linked, as a consequence of homologous integration, to regulatory sequences (e.g., an enhancer) of the endogenous APP gene so that the heterologous Arctic mutation gene is expressed under the transcriptional control of regulatory elements from the endogenous APP gene locus. Non-human hosts which are homozygous for such replacement alleles (i.e., a host chromosomal APP locus which encodes a cognate heterologous Arctic mutation APP gene product) may be produced according to methods described herein. Such homozygous non-human hosts generally will express a heterologous Arctic mutation APP protein but do not express the endogenous APP protein. Most usually, the expression pattern of the heterologous Arctic mutation APP gene will substantially mimic the expression pattern of the endogenous APP gene in the naturally-occurring (non-transgenic) non-human host. For example but not limitation, a transgenic mouse having human Arctic mutation APP gene sequences replacing the endogenous murine APP gene sequences and which are transcriptionally controlled by endogenous murine regulatory sequences generally will be expressed similarly to the murine APP in naturally occurring non-transgenic mice.

Generally, a replacement-type targeting construct is employed for homologous gene replacement. Double-crossover homologous recombination between endogenous APP gene sequences and homology clamps flanking the replacement region (i.e., the heterologous Arctic mutation APP encoding region) of the targeting construct result in targeted integration of the heterologous Arctic mutation APP gene segments. Usually, the homology clamps of the transgene comprise sequences which flank the endogenous APP gene segments, so that homologous recombination results in concomitant deletion of the endogenous APP gene segments and homologous integration of the heterologous gene segments. Substantially an entire endogenous APP gene may be replaced with a heterologous APP gene comprising the Arctic mutation by a single targeting event or by multiple targeting events (e.g., sequential replacement of individual exons). One or more

selectable markers, usually in the form of positive or negative selection expression cassettes, may be positioned in the targeting construct replacement region; it is usually preferred that selectable markers are located in intron regions of the heterologous replacement region.

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ES cells harboring a heterologous Arctic mutation APP gene, such as a replacement allele, may be selected in several ways. First, a selectable marker (e.g., neo, gpt, tk) may be linked to the heterologous Arctic mutation APP gene (e.g., in an intron or flanking sequence) in the targeting construct so that cells having a replacement allele may be selected for. Most usually, a heterologous APP gene targeting construct will comprise both a positive selection expression cassette and a negative selection expression cassette, so that homologously targeted cells can be selected for with a positive-negative selection scheme (Mansour et al. (1988) op.cit., incorporated herein by reference). Generally, a positive selection expression cassette is positioned in an intron region of the heterologous Arctic mutation APP gene replacement region, while a negative selection expression cassette is positioned distal to a homology clamp, such that double-crossover homologous recombination will result in the integration of the positive selection cassette and the loss of the negative selection cassette.

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20 Targeting Constructs

Several gene targeting techniques have been described, including but not limited to: co-electroporation, "hit-and-run", single-crossover integration, and double-crossover recombination (Bradley et al. (1992) Bio/Technology 10: 534). The invention can be practiced using essentially any applicable homologous gene targeting strategy known in the art. The configuration of a targeting construct depends upon the specific targeting technique chosen. For example, a targeting construct for singlecrossover integration or "hit-and-run" targeting need only have a single homology clamp linked to the targeting region, whereas a double-crossover replacement-type targeting construct requires two homology clamps, one flanking each side of the replacement region.

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For example and not limitation, a preferred embodiment is a targeting construct comprising, in order: (1) a first homology clamp having a sequence substantially identical to a sequence within about 3 kilobases upstream (i.e., in the direction opposite to the translational reading frame of the exons) of an exon of an endogenous APP gene, (2) a

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replacement region comprising a positive selection cassette having a pgk promoter driving transcription of a neo gene, (3) a second homology clamp having a sequence substantially identical to a sequence within about 3 kilobases downstream of said exon of said endogenous APP gene, and (4) a negative selection cassette, comprising a HSV tk promoter driving transcription of an HSV tk gene. Such a targeting construct is suitable for double-crossover replacement recombination which deletes a portion of the endogenous APP locus spanning said exon and replaces it with the replacement region having the positive selection cassette. If the deleted exon is essential for expression of a functional APP gene product, the resultant exon-deleted allele is functionally disrupted and is termed a null allele.

Targeting constructs of the invention comprise at least one APP homology clamp linked in polynucleotide linkage (i.e., by phosphodiester bonds) to a targeting region. A homology clamp has a sequence which substantially corresponds to, or is substantially complementary to, an endogenous APP gene sequence of a non-human host animal, and may comprise sequences flanking the APP gene.

Although no lower or upper size boundaries for recombinogenic homology clamps for gene targeting have been conclusively determined in the art, the best mode for homology clamps is believed to be in the range between about 50 basepairs and several tens of kilobases. Consequently, targeting constructs are generally at least about 50 to 100 nucleotides long, preferably at least about 250 to 500 nucleotides long, more preferably at least about 1000 to 2000 nucleotides long, or longer. Construct homology regions (homology clamps) are generally at least about 50 to 100 bases long, preferably at least about 100 to 500 bases long, and more preferably at least about 750 to 2000 bases long. It is believed that homology regions of about 7 to 8 kilobases in length are preferred, with one preferred embodiment having a first homology region of about 7 kilobases flanking one side of a replacement region and a second homology region of about 1 kilobase flanking the other side of said replacement region. The length of homology (i.e., substantial identity) for a homology region may be selected at the discretion of the practitioner on the basis of the sequence composition and complexity of the endogenous APP gene target sequence(s) and guidance provided in the art (Hasty et al. (1991) Mol. Cell. Biol. 11: 5586; Shulman et al. (1990) Mol. Cell. Biol. 10: 4466). Targeting constructs have at least one homology region having a sequence that substantially corresponds to, or is substantially complementary to, an endogenous APP gene sequence (e.g., an exon sequence, an

enhancer, a promoter, an intronic sequence, or a flanking sequence within about 3-20 kb of a APP gene). Such a targeting transgene homology region serves as a template for homologous pairing and recombination with substantially identical endogenous APP gene sequence(s). In targeting constructs, such homology regions typically flank the replacement region, which is a region of the targeting construct that is to undergo replacement with the targeted endogenous APP gene sequence (Berinstein et al. (1992) Mol. Cell. Biol. 12: 360). Thus, a segment of the targeting construct flanked by homology regions can replace a segment of an endogenous APP gene sequence by double-crossover homologous recombination. Homology regions and targeting regions are linked together in conventional linear polynucleotide linkage (5' to 3' phosphodiester backbone). Targeting constructs are generally double-stranded DNA molecules, most usually linear.

Without wishing to be bound by any particular theory of homologous recombination or gene conversion, it is believed that in such a double-crossover replacement recombination, a first homologous recombination (e.g., strand exchange, strand pairing, strand scission, strand ligation) between a first targeting construct homology region and a first endogenous APP gene sequence is accompanied by a second homologous recombination between a second targeting construct homology region and a second endogenous APP gene sequence, thereby resulting in the portion of the targeting construct that was located between the two homology regions replacing the portion of the endogenous APP gene that was located between the first and second endogenous APP gene sequences. For this reason, homology regions are generally used in the same orientation (i.e., the upstream direction is the same for each homology region of a transgene to avoid rearrangements). Double-crossover replacement recombination thus can be used to delete a portion of an endogenous APP gene and concomitantly transfer a non-homologous portion (e.g., a neogene expression cassette) into the corresponding chromosomal location. Double-crossover recombination can also be used to add a non-homologous portion into an endogenous APP gene without deleting endogenous chromosomal portions. However, doublecrossover recombination can also be employed simply to delete a portion of an endogenous APP gene sequence without transferring a non-homologous portion into the endogenous APP gene (see Jasin et al. (1988) Genes Devel. 2:1353). Upstream and/or downstream from the non-homologous portion may be a gene which provides for identification of whether a double-crossover homologous recombination has occurred; such a gene is typically the HSV tk gene which may be used for negative selection.

Typically, targeting constructs of the invention are used for functionally disrupting endogenous APP genes and comprise at least two homology regions separated by a non-homologous sequence which contains an expression cassette encoding a selectable marker, such as neo (Smith and Berg (1984) Cold Spring Harbor Symp. Quant. Biol. 4.9: 171; Sedivy and Sharp (1989) Proc. Natl. Acad. Sci. (U.S.A.) 86: 227; Thomas and Capecchi (1987) op.cit.). However, some targeting transgenes of the invention may have the homology region(s) flanking only one side of a nonhomologous sequence. Targeting transgenes of the invention may also be of the type referred to in the art as "hit-and-run" or "in-and-out" transgenes (Valancius and Smithies (1991) Mol. Cell. Biol. 11: 1402; Donehower et al. (1992) Nature 356: 215; (1991) J. NIH Res. 3: 59; which are incorporated herein by reference).

The positive selection expression cassette encodes a selectable marker which affords a means for selecting cells which have integrated targeting transgene sequences spanning the positive selection expression cassette. The negative selection expression cassette encodes a selectable marker which affords a means for selecting cells which do not have an integrated copy of the negative selection expression cassette. Thus, by a combination positive-negative selection protocol, it is possible to select cells that have undergone homologous replacement recombination and incorporated the portion of the transgene between the homology regions (i.e., the replacement region) into a chromosomal location by selecting for the presence of the positive marker and for the absence of the negative marker.

Preferred expression cassettes for inclusion in the targeting constructs of the invention encode and express a selectable drug resistance marker and/or a HSV thymidine kinase enzyme. Suitable drug resistance genes include, for example: gpt (xanthine-guanine phosphoribosyltransferase), which can be selected for with mycophenolic acid; neo (neomycin phosphotransferase), which can be selected for with 6418 or hygromycin; and DFHR (dihydrofolate reductase), which can be selected for with methotrexate (Mulligan and Berg (1981) Proc. Natl. Acad. Sci. (U.S.A.) 78: 2072; Southern and Berg (1982) J. Mol. Appl. Genet. 1: 327; which are incorporated herein by reference).

Selection for correctly targeted recombinants will generally employ at least positive selection, wherein a non-homologous expression cassette encodes and expresses a

functional protein (e.g., neo or gpt) that confers a selectable phenotype to targeted cells harboring the endogenously integrated expression cassette, so that, by addition of a selection agent (e.g., G418 or mycophenolic acid) such targeted cells have a growth or survival advantage over cells which do not have an integrated expression cassette.

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It is preferable that selection for correctly targeted homologous recombinants also employ negative selection, so that cells bearing only nonhomologous integration of the transgene are selected against. Typically, such negative selection employs an expression cassette encoding the herpes simplex virus thymidine kinase gene (HSV tk) positioned in the transgene so that it should integrate only by non-homologous recombination. Such positioning generally is accomplished by linking the HSV tk expression cassette (or other negative selection cassette) distal to the recombinogenic homology regions so that double-crossover replacement recombination of the homology regions transfers the positive selection expression cassette to a chromosomal location but does not transfer the HSV tk gene (or other negative selection cassette) to a chromosomal location. A nucleoside analog, gancyclovir, which is preferentially toxic to cells expressing HSV tk, can be used as the negative selection agent, as it selects for cells which do not have an integrated HSV tk expression cassette. FIAU may also be used as a selective agent to select for cells lacking HSV tk.

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In order to reduce the background of cells having incorrectly integrated targeting construct sequences, a combination positive-negative selection scheme is typically used (Mansour et al. (1988) op.cit., incorporated herein by reference).

Generally, targeting constructs of the invention preferably include: (1) a positive selection expression cassette flanked by two homology regions that are substantially identical to host cell endogenous APP gene sequences, and (2) a distal negative selection expression cassette. However, targeting constructs which include only a positive selection expression cassette can also be used. Typically, a targeting construct will contain a positive selection expression cassette which includes a neogene linked downstream (i.e., towards the carboxy-terminus of the encoded polypeptide in translational reading frame orientation) of a promoter such as the HSV tk promoter or the pgk promoter. More typically, the targeting transgene will also contain a negative selection expression cassette which includes an HSV tk gene linked downstream of a HSV tk promoter.

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It is preferred that targeting constructs of the invention have homology regions that are highly homologous to the predetermined target endogenous DNA sequence(s), preferably isogenic (i.e., identical sequence). Isogenic or nearly isogenic sequences may be obtained by genomic cloning or high-fidelity PCR amplification of genomic DNA from the strain of
5 non-human animals which are the source of the ES cells used in the gene targeting procedure.

To disrupt the murine APP gene, a targeting construct based on the design employed by Jaenisch and co-workers (Zijlstra, et al. (1989) op.cit.) for the successful disruption of the
10 mouse β 2-microglobulin gene can be used. The neomycin resistance gene (neo), from the plasmid pMC INE0 is inserted into the coding region of the target APP gene. The pMC INE0 insert uses a hybrid viral promoter/enhancer sequence to drive neo expression. This promoter is active in embryonic stem cells. Therefore, neo can be used as a selectable marker for integration of the knock-out construct. The HSV thymidine kinase (tk) gene is
15 added to the end of the construct as a negative selection marker against random insertion events (Zijlstra, et al., op.cit.).

Vectors containing a targeting construct are typically grown in *E. coli* and then isolated using standard molecular biology methods, or may be synthesized as oligonucleotides.
20 Direct targeted inactivation which does not require prokaryotic or eukaryotic vectors may also be done. Targeting transgenes can be transferred to host cells by any suitable technique, including microinjection, electroporation, lipofection, biolistics, calcium phosphate precipitation, and viral-based vectors, among others. Other methods used to transform mammalian cells include the use of Polybrene, protoplast fusion, and others
25 (See, generally, Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference).

For making transgenic non-human animals (which include homologously targeted
30 non-human animals), embryonal stem cells (ES cells) are preferred. Murine ES cells, such as AB-1 line grown on mitotically inactive SNL76/7 cell feeder layers (McMahon and Bradley (1990) Cell 62: 1073) essentially as described (Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach. E. J. Robertson, ed. (Oxford: IRL Press), p. 71-112) may be used for homologous gene targeting. Other suitable
35 ES lines include, but are not limited to, the E14 line (Hooper et al. (1987) Nature 326:

292-295), the D3 line (Doetschman et al. (1985) J. Embryol. Exp. Morph. 87: 27-45), and the CCE line (Robertson et al. (1986) Nature 323: 445-448). The success of generating a mouse line from ES cells bearing a specific targeted mutation depends on the pluripotency of the ES cells (i.e., their ability, once injected into a host blastocyst, to participate in embryogenesis and contribute to the germ cells of the resulting animal). The blastocysts containing the injected ES cells are allowed to develop in the uteri of pseudopregnant non-human females and are born as chimeric mice. The resultant transgenic mice are chimeric for cells having inactivated endogenous APP loci and are backcrossed and screened for the presence of the correctly targeted transgene(s) by PCR or Southern blot analysis on tail biopsy DNA of offspring so as to identify transgenic mice heterozygous for the inactivated APP locus. By performing the appropriate crosses, it is possible to produce a transgenic non-human animal homozygous for functionally disrupted APP alleles, and optionally also harboring a transgene encoding a heterologous APP polypeptide comprising the Arctic mutation. Such transgenic animals are substantially incapable of making an endogenous APP gene product but express the Arctic mutation heterologous APP.

Commercial Research and Screening Uses

Non-human animals comprising transgenes which encode Arctic mutation APP (and thus Arctic mutation A(3), can be used commercially to screen for agents having the effect of lowering A(3) production and/or accumulation. Such agents can be developed as pharmaceuticals for treating abnormal APP processing and/or Alzheimer's disease, amongst other neurodegenerative conditions. For example, the p53 knockout mice of Donehower et al. (1992) Nature 356:215 have found wide acceptance as commercial products for carcinogen screening and the like. The transgenic animals of the present invention exhibit abnormal APP processing and expression, and can be used for pharmaceutical screening and as disease models for neurodegenerative diseases and APP biochemistry. Such animals have many uses, including but not limited to identifying compounds that effect or affect A β processing; in one variation, the agents are thereby identified as candidate pharmaceutical agents. The transgenic animals can also be used to develop agents that modulate APP (or A β) expression and/or stability; such agents can serve as therapeutic agents to treat neurodegenerative diseases. The knockout animals of the invention can also serve as disease models for investigating APP-related pathological

conditions (e.g., Alzheimer's disease and the like). Such transgenic animals can be commercially marketed to researchers, among other uses.

Antibodies for Arctic Mutation APP

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Using APP polypeptides comprising the Arctic mutation, it is then possible to prepare antisera and monoclonal antibodies using, for example, the method of Kohler and Milstein ((1975) Nature 256:495). Such monoclonal antibodies could then form the basis of a diagnostic test for the presence of the Arctic mutation, among other uses.

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Arctic mutation APP polypeptides may be used to immunize an animal for the production of specific antibodies. These antibodies may comprise a polyclonal antiserum or may comprise a monoclonal antibody produced by hybridoma cells. For general methods to prepare antibodies, see Antibodies: A Laboratory Manual, (1988) E. Harlow and D. Lane, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., which is incorporated herein by reference.

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For example but not for limitation, a recombinantly produced fragment of the Arctic mutation APP695 polypeptide can be injected into a mouse along with an adjuvant so as to generate an immune response. Murine immunoglobulins which bind the recombinant fragment with a binding affinity of at least $1 \times 10^6 \text{ M}^{-1}$ can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells. The bank of hybridomas can be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an affinity of at least $1 \times 10^6 \text{ M}^{-1}$. More specifically, immunoglobulins that bind to the Arctic mutation APP polypeptide but have limited crossreactivity with a wild-type APP polypeptide are selected, either by preabsorption with wild-type APP or by screening of hybridoma cell lines for specific idiotypes that preferentially bind the Arctic mutation variant as compared to the wild-type.

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Figure legends

The present invention will now be further described with reference to the enclosed figures, in which

- 5 Figure 1. The metabolic pathways of APP. The metabolism of APP can follow two pathways, one non-amyloid-forming and one amyloid-forming. The last pathway generates intact A β and causes Alzheimer's disease, according to the amyloid hypothesis. In the non-amyloid-forming pathway A β is cleaved and no amyloid deposition are formed. Both pathways normally occur.

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- Figure 2. A scheme of the Swedish family in which the Arctic mutation was found. The following symbols are used: A square relates to a man and a circle relates to a woman. A slash over the square or the circle means that the person is deceased. A full square or circle means that the person has developed Alzheimer's disease. The symbol "+/-" means
15 that the person carries the Arctic mutation, whereas the symbol "-/-" means that the person does not carry the mutation.

- Figure 3a. The APP molecule with localization of the A β and p3 peptides, containing pathogenic intra-A β mutations. 3b. Pedigree showing the segregation of AD and of the
20 E693G mutation in the Arctic family, compatible with an autosomal dominant pattern of inheritance. The pedigree has been disguised to protect the confidentiality of family members.

- Figure 4. Plasma A β levels in the Arctic family. Plasma from nine mutation carriers and
25 eleven non-carriers in the family were analyzed by end-specific ELISAs. A significant decrease of A β 42 and A β 40 was observed in carriers of the Arctic mutation.

- Figure 5. Elution profiles showing A β 1-40wt (a-c) versus A β 1-40Arc (d-f) at 5 (a,d), 45 (b,e) and 125 (c,f) min of incubation. Accelerated protofibril (p) formation along with a
30 parallel decline in the monomeric/dimeric (m/d) A β levels could be observed for A β 1-40Arc (d-f) as compared to A β 1-40wt (a-c). Data is from one experiment, representative of four. Initial peptide concentrations were 143 μ M and 138 μ M for A β 1-40wt and A β 1-40Arc, respectively.

Figure 6. Kinetics of soluble forms of A β 1-40. Monomeric/dimeric (grey dots) and protofibrillar (black dots) a A β 1-40wt (88 μ M) and b A β 1-40Arc (92 μ M). Data is taken from one experiment, representative of three.

- 5 Figure 7. High magnification transmission electron micrographs (315 000 x) of negatively stained A β 1-40wt and A β 1-40Arc sedimented peptide samples. a A β 1-40wt protofibrils were seen with their typical curved appearance and with lengths of 30-60 nm. b A β 1-40Arc protofibrils were longer and appeared more rigid than the A β 1-40wt protofibrils. The diameters of the two peptide variants were similar (6-7 nm)(a, b). However, numerous
- 10 short A β 1-40Arc fibrils were also observed that exhibited larger diameters (ca 10-18 nm) resulting from intertwining of 2-3 fibrils (b). Scale bar 100 nm (a, b).

Examples

The following examples are provided for illustration and are not intended to limit the invention to the specific example provided.

5 Example 1

An APP mutation (E693G) in a family from northern Sweden, named the "Arctic" family, is identified, which spans over four generations (Fig. 3b). Clinical information was available on eleven affected cases in three generations. An autosomal dominant pattern of inheritance was seen in the family with a mean age of onset at 57 ± 2.9 years (range 54 to 61 years). Clinical examination, neuropsychological testing, brain imaging (computed tomography or magnetic resonance imaging) and EEG were used in evaluating patients according to DSM-IV criteria. Clinical history was typical for AD, with a slow insidious progression and decline in memory for recent events as the first presenting symptom. Signs of strokes or vascular lesions were not seen on brain imaging in seven investigated patients. The family was screened for mutations in exons 16 and 17 of the APP gene by single strand conformation polymorphism analysis (SSCP) (L. Forsell, L. Lannfelt, *Neurosci Lett* **184**, 90-93 (1995)). An abnormal mobility pattern was observed in exon 17. Sequencing revealed an A→G nucleotide substitution, representing a glutamic acid to a glycine substitution at APP codon 693 (E693G), corresponding to position 22 in the A β sequence. Venous blood was drawn into tubes containing EDTA and DNA was prepared according to standard procedures. SSCP was performed as described (17). To sequence exon 17 of the APP gene a 319 bp fragment was amplified with the following primers SEQ ID NO. 5: 5'-CCT CAT CCA AAT GTC CCC GTC ATT-3' and SEQ.ID.NO. 6 5'-GCC TAA TTC TCT CAT AGT CTT AAT TCC CAC-3'. The PCR products were purified with QIAquick PCR purification kit (Qiagen) prior to sequencing. Direct sequencing was performed in both 3' and 5' direction using the same primers and the BIG Dye cycle sequencing protocol (PE Biosystems) and were then analyzed on an ABI377 automated sequencer (PE Biosystems). The Arctic mutation was seen in one family and not in 56 controls or 254 cases with dementia. The mutation was further verified by restriction analysis, since it destroyed a MboII restriction site. The mutation was fully penetrant as no escapees were found. Two-point linkage analysis was performed between the mutation and affection status in the family with an age-dependent penetrance, giving a lod score of 3.66 at recombination fraction 0.00. Two-point lod score was calculated using Mlink from

the linkage package (version 5.1) at each of the following recombination fractions 0.00, 0.10, 0.20, 0.30 and 0.40 (q males=q females). A single-locus model with an autosomal dominant inheritance was assumed, which was compatible with the inheritance as it appeared in the pedigree. A cumulative age dependent penetrance was assigned from
5 the known ages of onset in the family. Individuals were put into different liability classes depending on the age at onset (affected) or age at last examination (unaffected). The disease gene frequency and the marker allele frequency were estimated to be 0.001 and the phenocopy rate was set to 0.0001.

10 17 members of the family have been investigated and eight of them carry the mutation. Of these eight, four have developed AD and the other four were below the age of onset. A lodscore of 2.32 was found by two-point linkage analysis with agedependent penetrance (Lathrop et al. (1984) PNAS 81:3443). 56 healthy controls were also investigated and none of them carried the Arctic mutation.

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Example 2

The family with the "Arctic" mutation was clinically and genealogically investigated. The family extends over five generations and originates from northern Sweden. Information on dementia, date of onset of the disease and its course, was obtained for nine affected
20 individuals by interviewing family members, and by collecting information from medical records, parish registers and historical archives. In this family the mean age of onset was 56.6 years and the mean duration of the disease was 7 years (n=5).

Early in the disease progression memory for recent events is impaired in Alzheimer's
25 disease, usually before deterioration of language, abstract reasoning, judgment and praxis. Often there is a lack of insight early in the course of the disease. Later, there is a global cognitive decline and motor impairment, usually with rigidity and gait disturbances. The first symptom in most cases in this family was an insidious loss of memory for recently acquired information. Symptoms before clinical manifestation of Alzheimer's
30 disease were decreased power of concentration and difficulties in handling stress situations. All affected individuals in generation IV had an early retirement pension because of the disease. The patients in generation IV were investigated by magnetic resonance imaging (MRI), computed tomography (CT) and electroencephalography (EEG) which confirmed the diagnosis of Alzheimer's disease. In four individuals CT and

MRI showed small infarcts and/or ischemic lesions, similar to what is seen in vascular dementia, without obvious neurological symptoms. The still living patients were all treated with cholinesterase inhibitors, with good results. Figure 2 presents a scheme of the family showing the presence of the Arctic mutation in 5 generations.

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Example 3

Pathogenic APP mutations have been shown to affect APP processing, as reflected in an increase of either total A β or A β 42 in the plasma of affected family members. The Arctic mutation is located in a region different from other AD-causing mutations, and

10 investigated as to whether the mutation manifested itself by affecting A β plasma levels. Plasma from nine mutation carriers, of which four were symptomatic, and eleven non-carriers in the family were analysed by well-characterized sandwich ELISA systems, specifically detecting A β 42 (BAN50/BC05) and A β 40 (BAN50/BA27) (N. Suzuki, et al., *Science* 264, 1336-1340 (1994)). The concentration of A β in plasma and in the

15 conditioned media was measured by the end specific ELISAs described previously (N. Suzuki, et al., *Science* 264, 1336-1340 (1994)). A β was captured with BAN50 and A β 40 and A β 42 were subsequently detected with BA27 and BC05, respectively. To reassure that the Arctic mutation did not change any of the antibody recognition sites A β 1-40wt and A β 1-40Arc peptides were tested and found to be recognized equally well. However, it was

20 observed that the antibodies BNT77 and 4G8, both raised against epitopes in the middle of the A β peptide, had reduced affinity for the Arctic peptide, thus they were not used. Furthermore, plasma was spiked with synthetic peptides, revealing that both A β Arc and A β wt peptides were recovered by ELISA to the same extent. The data obtained was analyzed by non-parametric Mann-Whitney analysis. The A β 42 plasma concentration was

25 11.7 ± 3.9 fmol/ml and 16.0 ± 5.6 fmol/ml in mutation carriers and non-carriers, respectively (Fig. 2), representing a 27% reduction of A β 42 in the mutation carriers ($p=0.04$). The A β 40 plasma concentration was 105 ± 22 fmol/ml and 141 ± 34 fmol/ml in mutation carriers and non-carriers, respectively (Fig. 4), representing a 26% reduction of A β 40 in the mutation carriers ($p=0.01$). The A β 42/40 ratio was calculated for each

30 individual, but no significant difference was found ($p=0.13$). In conclusion, concentrations of both A β 42 and A β 40 were unexpectedly and significantly reduced in individuals carrying the Arctic mutation.

Example 4

The effect of the Arctic mutation on A β formation was further investigated *in vitro* in transiently transfected HEK293 cells. APPwt was compared to the following mutations: Arctic (APP_{E693G}), Dutch (APP_{E693Q}), Italian (APP_{E693K}) and Flemish (APP_{A692G}). The locations of the mutations are illustrated in Fig. 3a. Constructs containing the Swedish double mutation (APP_{Swe}) and one APP mutation at codon 717 (APP_{V717F}), both with well-studied APP processing characteristics (J. Hardy, *Trends Neurosci.* 20, 154-159 (1997)), were used as positive controls. The mutations were introduced to APP695 cDNA in pcDNA3 using QuikChange™ Site-Directed Mutagenesis Kit according to the manufacturers instructions (Stratagene). The mutated constructs were verified by sequencing. For the ELISA measurements, HEK293 cells were seeded in six-well dishes and transfected with the different constructs using FuGENE™ 6 Transfection Reagent (Roche Diagnostics) according to the manufacturers instructions. 24 h after transfection, the cells were conditioned 48 h in OptiMEM containing 5% newborn calf serum. After withdrawal of the media for ELISA measurements, the APP expression in the cells were investigated by western blot using monoclonal antibody 22C11 (Roche Diagnostics). Media was conditioned and analyzed for A β levels by the same A β 42- and A β 40-specific sandwich ELISA systems as used for human plasma (M. Citron, et al. *Nature Med* 3, 67-72 (1997)). The A β 42 and A β 40 concentrations and A β 42/40 ratios are shown in Table 1.

Table 1 A β 42/40 ratio and A β 42 and A β 40 levels in conditioned media from transiently transfected HEK293 cells

APP constructs	A β 42/40 ratio (%) \pm SD	A β 42 \pm SD (fmol/ml)	A β 40 \pm SD (fmol/ml)
APPwt	9.6 \pm 0.7	13.8 \pm 1.0	144 \pm 6
Arctic (E693G)	7.5 \pm 0.5*	11.2 \pm 0.6	149 \pm 3
Dutch (E693Q)	6.6 \pm 0.6*	9.6 \pm 0.7	147 \pm 12
Italian (E693K)	6.4 \pm 0.6*	8.0 \pm 0.7	126 \pm 17
Flemish (A692G)	11.7 \pm 1.6*	27.0 \pm 2.0	232 \pm 25
Mock (vector only)	7.2 \pm 2.4	2.1 \pm 1.0	28 \pm 5

* P=0.004 in comparison to APPwt

Care was taken to obtain similar APP expression between different mutations and experiments, which resulted in almost identical APP levels (not shown). In accordance with previous reports (M. Citron, et al., *Nature* **360**, 672-674 (1992)), A β 42 and A β 40 peptide concentrations in media were increased 4-5 times by the Swedish mutation. Increased A β 42 levels were seen in media by the APP717 mutation, leading to an approximately three-fold increase in the A β 42/40 ratio, in agreement with data previously reported (N. Suzuki, et al., *Science* **264**, 1336-1340 (1994)). Strikingly, all mutations located at codon 693 (Arctic, Dutch and Italian) showed the same effect on A β levels: the A β 42 concentration was significantly lower in conditioned media from cells transfected with APP693 mutations, whereas A β 40 levels were similar to APPwt transfected cells. Consequently the A β 42/40 ratio showed a 22-33% reduction (Table 1). This finding was in contrast to the APP692 Flemish mutation, where increased levels of both A β 42 and A β 40 were found in conditioned media with a slight increase in A β 42/40 ratio, similar to previously reported (N. Suzuki, et al., *Science* **264**, 1336-1340 (1994)). All experiments were repeated three times, with similar results.

Example 5

The effects of the intra-A β mutations on A β secretion from transiently transfected cells were investigated. To increase the A β levels, the mutations were combined with the Swedish mutation. For this aim an ELISA assay was developed which selectively measures A β 40 and A β 42.

Table 2. The APP mutations investigated in this study, showing their position and pathology. All affect the APP processing, leading to altered A β production.

APP mutation	Position	Pathology
Swedish	β -secretase site (codon 670, 671)	AD
London	γ -secretase site (codon 717)	AD
Dutch	α -secretase site (codon 693)	Cerebral haemorrhage
Italian	α -secretase site (codon 693)	Cerebral haemorrhage
Flemish	α -secretase site (codon 692)	Cerebral haemorrhage/AD
Arctic	α -secretase site (codon 693)	AD

In vitro mutagenesis

The QuikChange™ Site-Directed Mutagenesis Kit (Stratagene) was used to mutagenize APP695-Swe (Lys670Asn, Met671Leu) cDNA, in the pcDNA3 vector, with the Dutch (Glu693Gln), Italian (Glu693Lys), Flemish (Ala692Gly), Arctic (Glu693Gly) and one of the

5 London (Val717Phe) mutation.

Transfection

Human Embryonic Kidney (HEK) 293 cells were transiently transfected with the double mutated APP constructs and with pcDNA3 vector containing the APP-Swe mutation only.

10 Conditioned media were collected for Enzyme Linked Immunosorbent Assay (ELISA) and the cells were lysated to be used for Western Blot.

ELISA

The collected cell media was subjected to sandwich ELISA using the capture antibody

15 6E10 (Senetek, Napa, CA), specific for the N-terminus of A β . A β 40 and A β 42 were detected by the endspecific polyclonal antibodies K12Ger and K19Ger, respectively, and then horseradish peroxidase conjugated to anti rabbit antibodies were added. Detection was made using the substrate orthophenylenediamine.

20 Western Blot

Western Blot was preformed on the cell lysates from the transfected HEK 293 cells.

22C11 (Roche) antibody specific for APP was used as a primary antibody and goat α -mouse IgG (Pierce) was used for detection.

25 By combining the Swedish mutation with the intra-A β mutations the A β production was increased and ELISA detection of A β 42 was facilitated (table 2). Importantly, this combination of mutations demonstrates no changes in A β 42/A β 40 ratios compared with previous results (Nilsberth *et al.*, submitted) using constructs with only the different intra-A β mutations.

30

Table 3. Results from th ELISA measurements. A β 40 and A β 42 levels in conditioned media from cells transiently transfected with APP695 containing the Swedish mutation only or in combination with the Arctic, Dutch, Italian, Flemish or London mutation. The transfections were performed in triplicates and measured in duplicate with an ELISA assay. A Mann-Whitney U-test was performed by comparing the Swedish mutation with the double mutations.

APP mutation(s)	A β 40 (fmol/ml)	P-value	A β 42 (fmol/ml)	P-value	A β 442/ A β 40 ratio (%)
Swedish	160 \pm 6.1		14.4 \pm 0.67		9.0
Swedish-Arctic	134 \pm 9.0	0.039	10.9 \pm 0.50	0.039	8.1
Swedish-Dutch	125 \pm 14.1	0.039	9.7 \pm 1.23	0.039	7.8
Swedish-Italian	137 \pm 7.3	0.039	9.2 \pm 0.28	0.039	6.7
Swedish-Flemish	261 \pm 5.1	0.039	29.1 \pm 1.23	0.039	11.1
Swedish-London	82 \pm 6.4	0.039	37.2 \pm 2.49	0.039	45

This study shows that constructs with the Arctic mutation containing also the Swedish mutation lead to significantly decreased extracellular levels of both A β 40 and A β 42.

10 **Example 6**

The effect of the single amino acid substitution (Glu22Gly) on amyloid fibrillization kinetics was investigated. Synthetic A β 1-40 was dissolved in physiological buffer and incubated for different periods of time. After centrifugation, the soluble A β in the supernatant, both low molecular weight (monomeric/dimeric) A β and protofibrils, were separated and analyzed using size exclusion chromatography (SEC) with UV detection at 214 nm. The morphology of the sedimented insoluble A β was visualized using negative stain and transmission electron microscopy (TEM) A β 1-40wt was purchased from Bachem, B ubendorf, Switzerland or Biosource International/QCB (Camarillo, CA, USA) and A β 1-40Arc from Biosource International/QCB. The peptides were trifluoroacetic salts. They were stored at -20 C. All other chemicals were of highest purity available. Samples of each peptide were incubated, without agitation, at 30 C in 50 mM Na₂HPO₄ · NaH₂PO₄ (pH 7.4) containing 0.1 M NaCl, for various time-points. Initial peptide concentrations were within the range of 88-143  M, and were similar for both peptides in each experiment. After centrifugation (17 900 x g for 5 min at 16 C) monomeric/dimeric and

protofibrillar A β 1-40, sampled from the supernatant, were separated using SEC. A Merck Hitachi D-7000 LaChrom HPLC system, having a diode array detector model L-7455, a L-7200 model autosampler and a model L-7100 pump, coupled to a Superdex 75 PC3.2/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden), was used for the

5 chromatographic separation and analysis. Samples were eluted at a flow rate of 0.08 ml/min (ambient temperature) using 50 mM Na₂HPO₄ NaH₂PO₄ (pH 7.4), 0.15 M NaCl. Chromatograms were obtained by measuring UV absorbance at 214 nm. Peak areas for monomeric/dimeric and protofibrillar A β were integrated using Merck-Hitachi Model D-7000 Chromatography Data Station Software. The mean of triplicate integrated peak
10 values from the SEC measurements were used to generate each data point shown in Fig. 5 and 6. In addition, a standard curve was produced by correlating integrated peak areas with peptide concentrations as determined by quantitative amino acid analysis. The concentrations of total (at t=0 h) and soluble peptides remaining in solution after centrifugation were calculated from the standard curve.

15

SEC analysis of freshly dissolved A β 1-40wt generated a single elution peak at a retention time of about 20 min (Fig. 3a). This peak represented the monomeric/dimeric forms of A β 1-40wt (D. M. Walsh, A. Lomakin, G. B. Benedek, M. M. Condron, D. B. Teplow, *J Biol Chem* 272, 22364-22372 (1997)). With increasing incubation time a second distinct
20 peak appeared in the gel-excluded fraction with a retention time of about 12 min. This earlier peak contained protofibrils (Fig. 6b, c), as verified by ultracentrifugation, negative stain and TEM of A β 1-40wt (data not shown), in line with previous findings (D. M. Walsh, A. Lomakin, G. B. Benedek, M. M. Condron, D. B. Teplow, *J Biol Chem* 272, 22364-22372 (1997)). Similar retention times were obtained for the A β 1-40Arc peptide (Fig. 6d-f).

25 However, A β 1-40Arc generated protofibrils much faster and in larger quantities than A β 1-40wt. Chromatograms from three early time-points of incubation illustrate this difference (Fig. 3). The monomeric/dimeric A β 1-40Arc peak declined in parallel with the growth of the protofibrillar peak (Fig. 6d-f). The maximum concentration (111 μ M) of A β 1-40Arc protofibrils was observed at 6.5 h.

30

Kinetic studies up to 48 h showed that A β 1-40wt generated a small quantity of protofibrils with a maximum concentration at 25 h (Fig. 5a). In contrast, a rapid and significant formation of protofibrils was seen within the first 5 h of incubation with a simultaneous

rapid decline in the concentration of the monomeric/dimeric A β 1-40Arc peptide (Fig. 5b). Despite the dramatic difference in monomeric/dimeric and protofibrillar kinetics between A β 1-40wt and A β 1-40Arc, there was no significant difference in fibrillization rate. This was verified in parallel kinetic studies monitoring the fluorescence of thioflavin T (data not
5 shown).

Example 7

A typical fibrillar morphology of A β 1-40Arc in sedimented samples from kinetic studies was confirmed by negative stain and TEM. A β peptide samples were prepared and
10 incubated as indicated for the kinetic studies, using higher peptide concentrations (617 μ M). After 8 days, aggregated A β species were sedimented using the same centrifugation parameters as described above. Buffer was removed and pelleted material was suspended in 50 μ l water using gentle sonication (2 x 6s). Eight μ l samples were applied to carbon stabilized Formvar film grids (Ted Pella, Inc., Redding, CA, USA). Samples were
15 negatively stained with 8 μ l uranyl acetate (1%) (E. Merck, Darmstadt, Germany). Four grids were prepared for each sample and examined using a Philips CM10 TEM. Samples from pellets sedimented during the kinetic experiments were also examined. Similar to the sedimented A β 1-40wt, large mesh-works of A β could be seen in these preparations. Protofibrils could also be discerned in the sedimented samples (Fig. 7a, b). The A β 1-
20 40Arc protofibrils were longer and less curved compared to the A β 1-40wt protofibrils. Inter-twining of several fibrils was more common in the A β 1-40Arc preparations, resulting in larger fibril diameters.

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SEQUENCE LISTING

SEQ.ID.NO.1 arctic mutant APP amino acid sequence (3 letter code)

5 Met Leu Pro Gly Leu Ala Leu Leu Leu Ala Ala Trp Thr Ala Arg
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
10
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu

15 Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
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Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu

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Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu

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Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu

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Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile

Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
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Cys Met Ala Val Cys Gly Ser Ala Met Ser Gln Ser Leu Leu Lys Thr

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Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp

Glu Asn Glu His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala
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Lys His Arg Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala

Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile

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Glu Arg Gln Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met

Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu
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Gln Val Met Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser

Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp

5 Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val

Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala

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Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp

20 Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu

Val Phe Phe Ala Gly Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly

Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu

25 Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val

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SEQ.ID.NO.2 wt APP amino acid sequence (1letter code)

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SEQ.ID.NO.3 arctic mutant APP nucleic acid sequence

15

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 3361 acattaaata atcgaagtaa tttgggtgg ggagaagagg cagattcaat tttcttaac
 3421 cagtctgaag ttcatattat gatacaaaag aagatgaaaa tggaagtggc aatataaggg
 3481 gatgaggaag gcatgcctgg acaaaccctt ctttaagat gtgtcttcaa ttgtataaa
 5 3541 atgggtttt catgtaaata aatacattct tggaggagc 3579

SEQ.ID.NO.4 wt APP nucleic acid sequence

1 agtttctcg gcagcggtag gcgagagcac gcggaggagc gtgcgcgggg gccccgggag
 10 61 acggcggcgg tggcggcgcg ggcagagcaa ggacgcggcg gatccactc gcacagcagc
 121 gcactcggtg ccccgcgtag ggtcgcgatg ctgcccgggt tggcactgct cctgctggcc
 181 gcctggacgg ctggggcgct ggaggtagcc actgatggta atgctggcct gctggctgaa
 241 cccagattg ccatgttctg tggcagactg aacatgcaca tgaatgtcca gaatgggaag
 301 tgggattcag atccatcagg gacaaaaacc tgcattgata ccaaggaagg catcctgcag
 15 361 tattgccaag aagtctaccc tgaactgcag atcaccaatg tggtagaagc caaccaacca
 421 gtgaccatcc agaactgggt caagcggggc cgcaagcagt gcaagaccca tcccacttt
 481 gtgattccct accgctgctt agttggtgag ttgttaagt atgcccttct cgttctgac
 541 aagtgcaaat tcttacacca ggagaggatg gatgtttcg aaactcatct tcactggcac
 601 accgtcgcca aagagacatg cagtgagaag agtaccact tgcattgata cggcatgttg
 20 661 ctgccctcg gaattgaca gttccgaggg gtagagttg tgtgtgccc actggctgaa
 721 gaaagtgaca atgtggattc tgctgatgag gaggaggatg actcggtatg ctggtggggc
 781 ggagcagaca cagactatgc agatgggagt gaagacaaag tagtagaagt agcagaggag
 841 gaagaagtgg ctgagggtga agaagaaga gccgatgatg acgaggacga tgaggatgtt
 901 gatgaggtag aggaagaggc tgaggaaccc tacgaagaag ccacagagag aaccaccagc
 25 961 attgccacca ccaccaccac caccacagag tctgtggaag aggtggttcg agaggtgtgc
 1021 tctgaacaag ccgagacggg gccgtgccga gcaatgatct cccgctggta ctttgatgtg
 1081 actgaagggg agtgtgcccc attcttttac ggcggaatgt gcggcaaccg gaacaacttt
 1141 gacacagaag agtactgcat ggccgtgtgt ggcagcgcca tgcctcaaag ttactcaag
 1201 actaccagag aacctctgc ccgagatcct gttaaacttc ctacaacagc agccagtacc
 30 1261 cctgatgccg ttgacaagta tctcagagaca cctggggatg agaatgaaca tgcccatttc
 1321 cagaaagcca aagagaggct tgaggccaag caccgagaga gaatgtcca ggcatgaga
 1381 gaatgggaag aggcagaacg tcaagcaaag aactgccta aagctgataa gaaggcagtt
 1441 atccagcatt tccaggagaa agtggaaatc ttggaacagg aagcagcaa cgagagacag
 1501 cagctggtgg agacacacat ggccagagtg gaagccatgc tcaatgaccg ccgcccgttc
 35 1561 gccctggaga actacatcac cgctctcag gctgttcctc ctggcctcg tcacgtgttc

1621 aatatgctaa agaagtatgt ccgcgacagaa cagaaggaca gacagcacac cctaaagcat
1681 ttcgagcatg tgcgcatggt ggatcccaag aaagccgctc agatccggtc ccaggttatg
1741 acacacctcc gtgtgattta tgagcgcatg aatcagtcct tctccctgct ctacaacgtg
1801 cctgcagtgg ccgaggagat tcaggatgaa gttgatgagc tgcttcagaa agagcaaaac
5 1861 tattcagatg acgtcttggc caacatgatt agtgaaccaa ggatcagtta cggaaacgat
1921 gctctcatgc catctttgac cgaaacgaaa accaccgtgg agctccttcc cgtgaatgga
1981 gagttcagcc tggacgatct ccagccgtgg cattcttttg gggctgactc tgtgccagcc
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2101 actcgaccag gttctgggtt gacaaatatc aagacggagg agatctctga agtgaagatg
10 2161 gatgcagaat tccgacatga ctcaggatat gaagttcatc atcaaaaatt ggtgttctt
2221 gcagaagatg tgggttcaaa caaaggtgca atcattggac tcatggtggg cgggtgtgtc
2281 atagcgacag tgatcgatc caccttggtg atgctgaaga agaaacagta cacatccatt
2341 catcatggtg tgggtggagg tgacgccgct gtcacccagc aggagcgcca cctgtccaag
2401 atgcagcaga acggctacga aaatccaacc tacaagttct ttgagcagat gcagaactag
15 2461 acccccgcca cagcagcctc tgaagttgga cagcaaaacc attgcttcac tacccatcgg
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2641 agtaatgtat tctatctctc ttacatttt ggtctctata ctacattatt aatgggtttt
2701 gtgtactgta aagaatttag ctgtatcaaa ctagtgcag aatagattct ctctgatta
20 2761 ttatcacat agccccttag ccagttgtat attattcttg tggtttgta cccaattaag
2821 tctacttta catatgcttt aagaatcgat gggggatgct tcatgtgaac gtgggagtc
2881 agctgcttct ctgctaag tattccttc ctgactacta tgcatttaa agttaaacat
2941 tttaagtat ttcagatgct ttgagagat tttttcca tgactgcatt ttactgtaca
3001 gattgctgct tctgctatat ttgtgatata ggaattaaga ggatacacac gttgtttct
25 3061 tcgtgcctgt ttatgtgca cacattagc attgagactt caagctttc tttttgtc
3121 cacgtatctt tgggtcttg ataaagaaaa gaatccctgt tcattgtaag cacttttacg
3181 gggcgggtgg ggaggggtgc tctgctggtc ttcaattacc aagaattctc caaaacaatt
3241 ttctgcagga tgattgtaca gaatcattgc ttatgacatg atcgcttct acactgtatt
3301 acataaataa attaaataaa ataaccgagg gcaagacttt tcttgaagg atgactacg
30 3361 acattaaata atcgaagtaa tttgggtgg ggagaagagg cagattcaat tttcttaac
3421 cagtctgaag ttcattht gatacaaaag aagatgaaaa tggaagtggc aatataaggg
3481 gatgaggaag gcatgcctgg acaaacctt ctttaagat gtgtctcaa ttgtataaa
3541 atggtgttt catgtaata aatacattct tggaggagc

SEQ.ID.NO.5	5'-CCT CAT CCA AAT GTC CCC GTC ATT-3'
SEQ.ID.NO.6	5'-GCC TAA TTC TCT CAT AGT CTT AAT TCC CAC-3'
SEQ.ID.NO.7	Val Phe Phe Ala Gly Asp Val Gly
SEQ.ID.NO.8	aattggtgtt cttgcagga gatgtgggtt caaacaagg
5 SEQ.ID.NO.9	gtttcaagg tgttcttgc
SEQ.ID.NO.10	ggaaacatgc agtcaagttt acc
SEQ.ID.NO.11	cctcatcaa atgtcccgt catt
SEQ.ID.NO.12	gcctaattct ctcatagtct taattccac

Claims

1. Use of an isolated nucleic acid sequence, which encodes the amino acid sequence of SEQ.ID.NO.1 including the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid sequence encodes glycine at codon 693, for detecting a mutation
- 5 in a gene coding for amyloid precursor protein (APP) indicating Alzheimer's disease (AD) in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said mutation, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.
- 10 2. Use of an isolated subfragment of a nucleic acid sequence, which encodes the amino acid sequence of SEQ.ID.NO.1, wherein the subfragment has between 10 and 40 nucleotides and encodes an amino acid sequence including the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid sequence encodes glycine at codon 693, for detecting a mutation in a gene coding for amyloid precursor
- 15 protein (APP) indicating Alzheimer's disease (AD) in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said mutation, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.
- 20 3. Use according to claim 1 or 2, wherein the A β 42 and/or A β 40 peptide levels in the plasma of said subject carrying said arctic mutation are decreased at least 10-30% compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.
4. Use according to any of claims 1-3, wherein the A β 42 and/or A β 40 peptide levels in the
- 25 plasma of said subject carrying said arctic mutation are decreased at least 20% compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.
5. Use according to any of claims 1-4, wherein the A β 42 and/or A β 40 peptide levels in the plasma of said subject carrying said arctic mutation are decreased at least 25% compared
- 30 to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.

6. Use according to any of claims 1-5, wherein said AD is further characterised by accelerated formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) compared to protofibrill formation of wild type A β peptides.
- 5 7. Use according to claim 6, wherein the formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) is accelerated at least 2-10 times compared to protofibrill formation of wild type A β peptides.
8. Use according to claim7, wherein the formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) is accelerated at least 5 times compared to protofibrill formation of wild type A β peptides.
- 10 9. Use according to any of claims1-8, for diagnosing or prognosing said AD.
- 15 10. Use according to any of claims1-9, for treating or preventing said AD.
11. Use according to any of claims1-10 of an isolated nucleic acid sequence complementary to a nucleic acid sequence or to a subfragment according to any of claims1-10.
- 20 12. Use according to any of claims1-11, wherein said nucleic acid sequence is selected from the group consisting of an isolated native, cloned recombinant or synthetic nucleic acid sequence.
- 25 13. Use according to any of claims1-12, wherein said nucleic acid sequence is labeled with a detectable moiety.
14. Use according to any of claims1-13 for detecting in a subject an increased likelihood of developing or transmitting to future generations said AD, said method comprising
- 30 analyzing a sample of DNA or RNA of said subject to determine the presence or absence of a mutation in a gene encoding the amino acid sequence of SEQ.ID.NO.2 wherein the nucleotides encoding codon 693 of said amino acid sequence are mutated to encode glycine at codon 693, the presence of such a mutation indicating an increased likelihood of developing or transmitting to future generations said AD.

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15. Use according to any of claims 1-14 for diagnosing said AD or a predisposition to develop said AD in an subject, comprising the step of determining the presence of a mutation in a gene encoding the amino acid sequence of SEQ.ID.NO.2 wherein the nucleotides encoding codon 693 of said amino acid sequence are mutated to encode
5 glycine at codon 693, or its normal complementary DNA or RNA sequences, in a sample of the DNA or RNA of such subject.

16. Use according to claim 14 or 15, wherein said determination comprises:
(a) providing a probe or primer comprising a subfragment of an isolated nucleic acid
10 sequence which encodes the amino acid sequence of SEQ.ID.NO.1, wherein the subfragment has between 10 and 40 nucleotides and encodes an amino acid sequence including the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid encodes glycine at codon 693,
(b) exposing said probe or primer to said sample of DNA or RNA of said subject; and
15 (c) detecting hybridization of said probe or primer to said DNA or RNA sample, thereby determining the presence of said mutation in said gene.

17. Use according to claim 14 or 15, wherein said determination comprises
(a) providing a probe or primer comprising a subfragment of an isolated nucleic acid
20 sequence which encodes the amino acid sequence of SEQ.ID.NO.1, wherein the subfragment has between 10 and 40 nucleotides and encodes an amino acid sequence including the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid encodes glycine at codon 693 ,
(b) combining said probe or primer with a second primer and said sample of DNA or RNA
25 of said subject, under conditions permitting amplification of a DNA or RNA template comprising said mutation in said gene; and
(c) detecting amplification of said DNA or RNA template, thereby determining the presence of said mutation in said gene.

30 18. Use according to any of claims 14-17, wherein the DNA or RNA sample is extracted from a body fluid of said subject comprising blood, lymph, cerebrospinal fluid, urin or sputum or from a specimen comprising tissue sample, brain substance or feeces.
19. Use according to any of claims 14-18, wherein said subject is a human.

20. A subfragment of an isolated nucleic acid sequence which encodes the amino acid sequence of SEQ.ID.NO.1, wherein the subfragment has between 10 and 40 nucleotides and encodes an amino acid sequence including the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid encodes glycine at codon 693.

5

21. A subfragment according to claim 20, wherein said nucleic acid sequence is an isolated native, cloned recombinant or synthetic nucleic acid sequence.

23. An isolated nucleic acid sequence which is complementary to a subfragment
10 according to claim 20 or 21.

24. An isolated nucleic acid or subfragment according to any of claims 20-23, labeled with a detectable moiety.

15 25. An isolated nucleic acid or subfragment according to claim 24, wherein the label is capable of emitting radiation.

26. An isolated nucleic acid or subfragment according to claim 25, wherein said label is selected from the group comprising ^{125}I , ^{32}P , ^{33}P , ^{35}S .

20

27. An isolated nucleic acid or subfragment according to claim 24, wherein said label is a component of an enzymatic reaction.

28. An isolated and purified nucleic acid sequence which encodes the amino acid
25 sequence of SEQ.ID.NO.1, isolated from a human subject with AD, said subject carrying a mutation in a gene coding for amyloid precursor protein (APP) that corresponds to the arctic mutation APP as shown in SEQ.ID.NO.1, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said arctic mutation, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier
30 subject.

29. A recombinant vector operable in a mammalian cell comprising a nucleic acid sequence or a subfragment of a nucleic acid sequence according to any of claims 20-28

35 30. A host cell comprising the recombinant vector of claim 29.

31. An immortalised mammalian cell line comprising a nucleic acid sequence or a subfragment of a nucleic acid sequence according to any of claims 20-28, or a vector according to claim 30.

5

32. A reagent for diagnosing, prognosing, treating or preventing AD in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, comprising a nucleic acid
10 sequence or a subfragment of an isolated nucleic acid sequence which encodes the amino acid sequence of SEQ.ID.NO.1, wherein the subfragment has between 10 and 40 nucleotides and encodes an amino acid sequence including the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid encodes glycine at codon 693.

15

33. Use of a reagent according to claim 32 for the diagnosis, prognosis, treatment or prevention of a AD in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-
20 carrier subject.

34. Use according to claim 33, wherein said subject is human.

35. The manufacture of a reagent according to claim 32.

25

36. A substantially purified polypeptide corresponding to an arctic mutation APP, or a splice variant thereof, said mutation comprising amino acid substitution to glycine at positions 693 of SEQ.ID.NO.1.

30 37. A recombinant vector comprising a nucleic acid sequence which encodes arctic mutation APP, or a splice variant thereof, comprising amino acid substitution to glycine at positions 693 of SEQ.ID.NO.1.

38. An isolated human neuronal cell comprising a recombinant vector according to claim
35 37, wherein the expression of arctic mutation APP is operably linked to regulatory

elements that are functional in said cell and wherein said cell expresses said arctic mutation APP.

39. A process for recombinantly producing a mutant human amyloid precursor protein
5 (APP) corresponding to the amino acid sequence of arctic mutation APP as shown in
SEQ.ID.NO.1, wherein amino acid 693 is a glycine, said arctic mutation APP being
characterised by yielding mutant A β peptides (40Arc and/or 42Arc) after processing,
which are characterised by displaying accelerated formation of protofibrils compared to
protofibrill formation of wild type A β , said process comprising culturing a host cell
10 according to claim 30 or 31 under conditions whereby the arctic mutation APP is
expressed, and isolating the arctic mutation APP therefrom.

40. A substantially purified polypeptide according to claim 36, which is isolated from
patients with AD or a predisposition for AD, said AD beeing characterised by decreased
15 A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in
the gene coding for APP, compared to A β 42 and/or A β 40 petide levels in the plasma of a
non-carrier subject.

41. A composition comprising the substantially purified polypeptide according to claim 36
20 or 40.

42. An antibody obtained by means of an immune response to exposure to a substantially
purified polypeptide according to claim 36 or 40.

25 43. An antibody according to claim 42, wherein the antibody is polyclonal.

44. An antibody according to claim 42 or 43, wherein the immune response is produced in
a mammal selected from the group consisting of rabbits, goats and mice.

30 45. An antibody according to claim 42, wherein the antibody is monoclonal.

46. An antibody according to claim 42 or 45, wherein the immune response is produced in
murine-murine hybridoma cells.

47. Use of an antibody according to any of claims 42-46 for the diagnosis of AD in a human subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.

5

48. A reagent for use in the diagnosis of AD in a human subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, comprising an antibody according to
10 any of claims 42-46.

49. A diagnostic method for determining the presence of AD in a human subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or
15 A β 40 peptide levels in the plasma of a non-carrier subject, by detecting the presence of arctic mutation APP in the patient through an immunoassay, comprising the steps of:
a. combining a sample of the human subject's body fluid suspected of containing arctic mutation APP with antibodies according to any of claims 42-46,
b. monitoring the combination of step a. to determine whether said antibodies have bound
20 to said arctic mutation APP in an immunological reaction, thereby indicating that said patient has said AD.

50. A diagnostic method according to claim 49, wherein said combining step further includes adding a known quantity of labelled wild type APP, whereby a competitive
25 immunoassay is established.

51. A diagnostic method according to claim 50, wherein said label is capable of emitting radiation.

30 52. A diagnostic method according to claim 51, wherein said label is selected from the group consisting of ^{125}I , ^{32}P , ^{33}P , ^{35}S .

53. A diagnostic method according to claim 50, wherein said label is a component of an enzymatic reaction.

54. An immunogenic peptide fragment of a substantially purified polypeptide isolated from patients with AD, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, comprising a linear sequence of amino acids corresponding to a subfragment of SEQ.ID.NO. 1, wherein the subfragment includes the nucleotides encoding codon 693 of said amino acid sequence encoding glycine at codon 693, said linear sequence being immunogenic so as to induce the production of active antibodies specific to arctic mutation APP.
55. An immunogenic peptide fragment according to claim 54, wherein the peptide fragment is about 10 amino acids in length.
56. A composition comprising a peptide fragment according to claim 54 or 55 solubilized in a basic guanidine solution.
57. A composition according to claim 56 coupled to an immunogenic carrier.
58. A diagnostic reagent comprising antibodies obtained by means of an immune response to exposure to a peptide fragment according to claim 54 or 55 or a composition according to claims 56 or 57.
59. The diagnostic reagent according to claim 58, wherein the antibodies are polyclonal.
60. The diagnostic reagent according to claim 58 or 59, wherein the immune response is produced in a mammal selected from the group consisting of rabbits, goats and mice.
61. The diagnostic reagent according to claim 58, wherein the antibodies are monoclonal.
62. The diagnostic reagent according to claim 58 or 61, wherein the immune response is produced in murine-murine hybridoma cells.
63. Use of a diagnostic reagent according to any of claims 58-62 for diagnosis, prognosis, treatment or prevention of AD in a human subject, said AD being

characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.

- 5 64. A diagnostic method for determining the presence of AD in a human subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, comprising the steps of:
- a. combining a sample of body fluid from the patient with a diagnostic reagent according
- 10 to any of claims 58-62,
- b. monitoring the combination of step a. to determine whether said antibodies have bound to arctic mutation APP in an immunological reaction, thereby indicating that said subject has said Alzheimer's Disease.
- 15 65. A diagnostic method according to claim 64, wherein said combining step further includes adding a known quantity of the peptide fragment according to claim 54 or 55 which has been labelled.
66. A diagnostic method according to claim 65, wherein said label is capable of emitting
- 20 radiation.
67. A diagnostic method according to claim 66, wherein said label is selected from the group consisting of ^{125}I , ^{32}P , ^{33}P , ^{35}S .
- 25 68. A diagnostic method according to claim 65, wherein said label is a component of an enzymatic reaction.
69. A diagnostic method for determining the presence or predisposition for AD in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in
- 30 the plasma of a subject carrying an arctic mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, comprising detecting the presence of arctic mutation APP in the patient through an immunoassay, comprising the steps of:
- a. combining a sample of the patient's tissue suspected of containing arctic mutation APP
- 35 with antibodies specific to a peptide fragment according to claim 54 or 55,

- b. monitoring the combination of step a. to determine whether said antibodies have bound to said arctic mutation APP in an immunological reaction, thereby indicating that said patient has said AD.
- 5 70. A diagnostic method according to claim 69, wherein said combining step further includes adding a known quantity of the peptide fragment according to claim 54 or 55 which has been labelled.
71. A diagnostic method according to claim 70, wherein said label is capable of emitting
10 radiation.
72. A diagnostic method according to claim 71, wherein said label is selected from the group consisting of ^{125}I , ^{32}P , ^{33}P , ^{35}S .
- 15 73. A diagnostic method according to claim 70, wherein said label is a component of an enzymatic reaction.
74. A transgenic rodent comprising a diploid genome comprising a transgene encoding a heterologous APP polypeptide having the arctic mutation wherein the amino acid residue
20 at a position corresponding to position 639 in human APP717 is glycine, wherein the transgene is expressed to produce a human APP polypeptide having the arctic mutation.
75. A transgenic rodent according to claim 74, wherein said polypeptide is processed to 40Arc and/or 42Arc in a sufficient amount to be detectable in a brain homogenate of said
25 transgenic rodent.
76. A transgenic rodent according to claim 74 or 75, wherein the animal is murine.
77. A transgenic rodent according to any of claims 74-76, wherein the transgene is
30 nonhomologously integrated.
78. A transgenic rodent according to any of claims 74-76, wherein the transgene is homologously integrated.

79. A transgenic rodent according to any of claims 74-78, wherein expression of said arctic mutation APP is under the control of a promoter sequence different from the promoter sequence controlling the transcription of the endogenous coding sequence for wild type APP.

5

80. A transgenic rodent according to claim 79, wherein the heterologous APP polypeptide having the arctic mutation is expressed under the transcriptional control of a neural-specific enolase promoter.

10 81. A transgenic rodent according to claim 79 or 80, wherein said promoter sequence controlling expression of said arctic mutation APP is inducible.

82. Use of a transgenic rodent according to any of claims 74-81, for modelling AD, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of
15 a subject carrying a mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject.

83. Use of a transgenic rodent according to any of claims 74-81, for modelling AD, said AD being characterised by accelerated formation of protofibrils comprising mutated A β
20 peptides (40Arc and/or 42Arc) compared to protofibril formation of wild type A β peptides.

84. Use of a transgenic rodent according to claim 82 or 83 for evaluating compositions that are likely to constitute a part of a pharmaceutical preparation for treating and/or preventing AD.

25

85. A method for identifying a specific variation of AD in a human subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying a mutation in the gene coding for APP, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, comprising detecting the presence or
30 absence of a mutation in the gene encoding the amino acid sequence of SEQ.ID.NO.2, wherein the nucleotides encoding codon 693 of said amino acid sequence are mutated to encode glycine at codon 693.

86. A method according to claim 85, wherein such subject is presymptomatic for
35 Alzheimer Disease and the presence of said mutation in the amino acid sequence of

SEQ.ID.NO.2, or in its complementary DNA or RNA sequences indicates an increased likelihood of developing or transmitting to future generations AD.

87. A method according to claim 85 or 86, wherein the presence of said mutation in the
5 amino acid sequence of SEQ.ID.NO.2, or in its complementary DNA or RNA sequences is determined by obtaining sample DNA or RNA from said individual in single stranded form, hybridizing the single stranded sample DNA or RNA with an oligonucleotide probe comprising the nucleotides encoding codon 693 of said amino acid sequence, wherein the nucleic acid encodes glycine at codon 693, or its corresponding RNA base sequence, or
10 their complementary DNA or RNA sequences, and analyzing resulting hybrids for homology.

88. A method according to claim 87, wherein said oligonucleotide probe consists of less than 50 nucleotides.
15

89. A method according to claim 88, wherein said oligonucleotide probe consists essentially of less than 30 nucleotides.

90. A method for testing a function related Alzheimer's disease (AD) in a subject, said AD
20 being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, the method comprising providing a transgenic animal according to any of claims 74-81 and testing the transgenic animal for alterations in said function.

25 91. A method according to claim 90, wherein said function is selected from behavioral functions associated with a neuropathological condition.

92. A method according to any of claims 90 and 91 wherein the function is early dementia.

30 93. A method according to claim 92 wherein the early dementia corresponds to dementia in a human before the age of 50 years, such as before 40 years such as before the age of 30 years.

94. A method for testing a treatment for a condition related to decreased A β 42 and/or
35 A β 40 peptide levels in the plasma of a subject, compared to A β 42 and/or A β 40 peptide

levels in the plasma of a non-carrier subject, the method comprising providing a transgenic animal according to any of claims 74-81, exposing said transgenic animal to the treatment and evaluating the effect of the treatment.

Abstract

- The present invention relates to the use of an isolated nucleic acid characteristic of human amyloid precursor protein (APP) 770 including a glycine substitution at codon 693, that corresponds to arctic mutation APP, for diagnosing or predicting a predisposition to
- 5 familial Alzheimer's disease (AD) in a subject, said AD being characterised by decreased A β 42 and/or A β 40 peptide levels in the plasma of a subject carrying said arctic mutation, compared to A β 42 and/or A β 40 peptide levels in the plasma of a non-carrier subject, and by accelerated formation of protofibrils comprising mutated A β peptides (40Arc and/or 42Arc) compared to protofibril formation of wild type A β peptides.
- 10 Also provided is a polypeptide corresponding to the arctic mutation APP and antibodies generated against said polypeptide. Further, the present invention provides a transgenic non-human mammal comprising a gene encoding amyloid precursor protein mutated at codon 693 in such a way that this codon encodes glycine. Finally, the present invention leads to possible therapeutic intervention using drugs targeted at preventing protofibril
- 15 formation.

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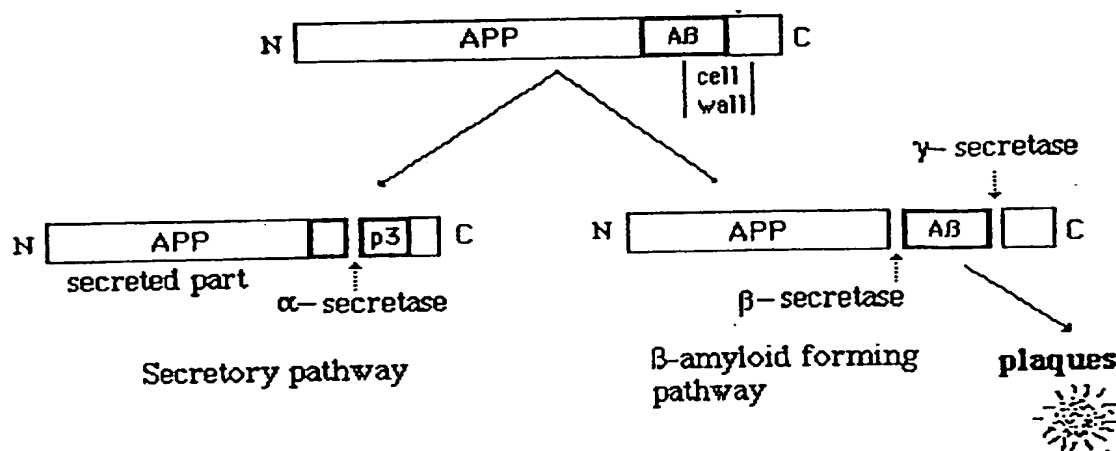
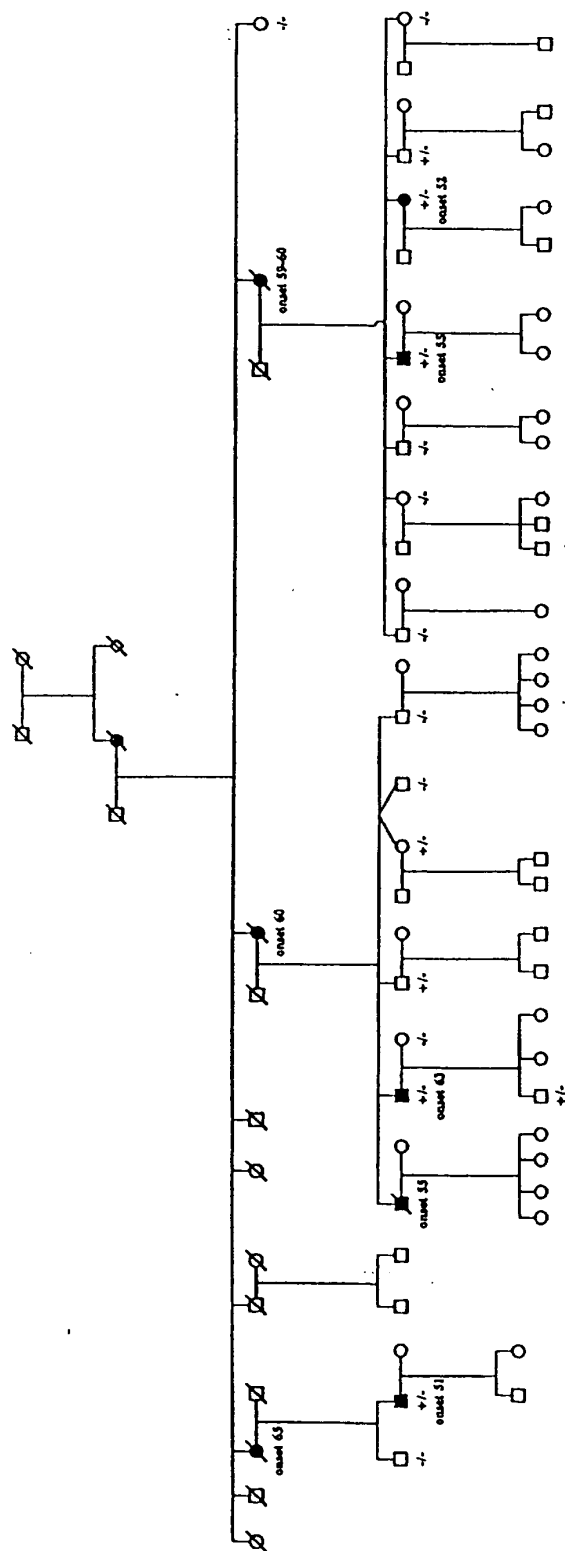


Fig. 1

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The Arctic APP mutation family



- male, unaffected, dead
- male, affected, dead
- male, unaffected, living
- male, affected, living
- female, unaffected, dead
- female, affected, dead
- female, unaffected, living
- female, affected, living
- mutation carrier
- normal, non-mutation carrier

Fig. 2

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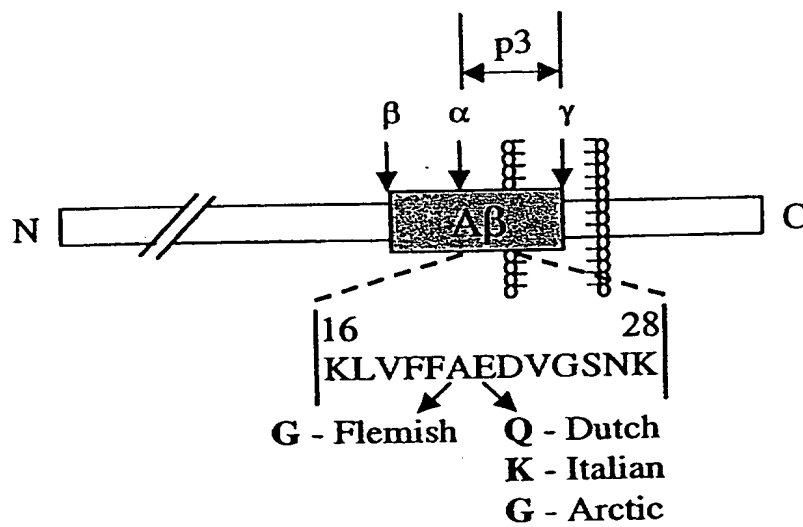


Fig. 3A

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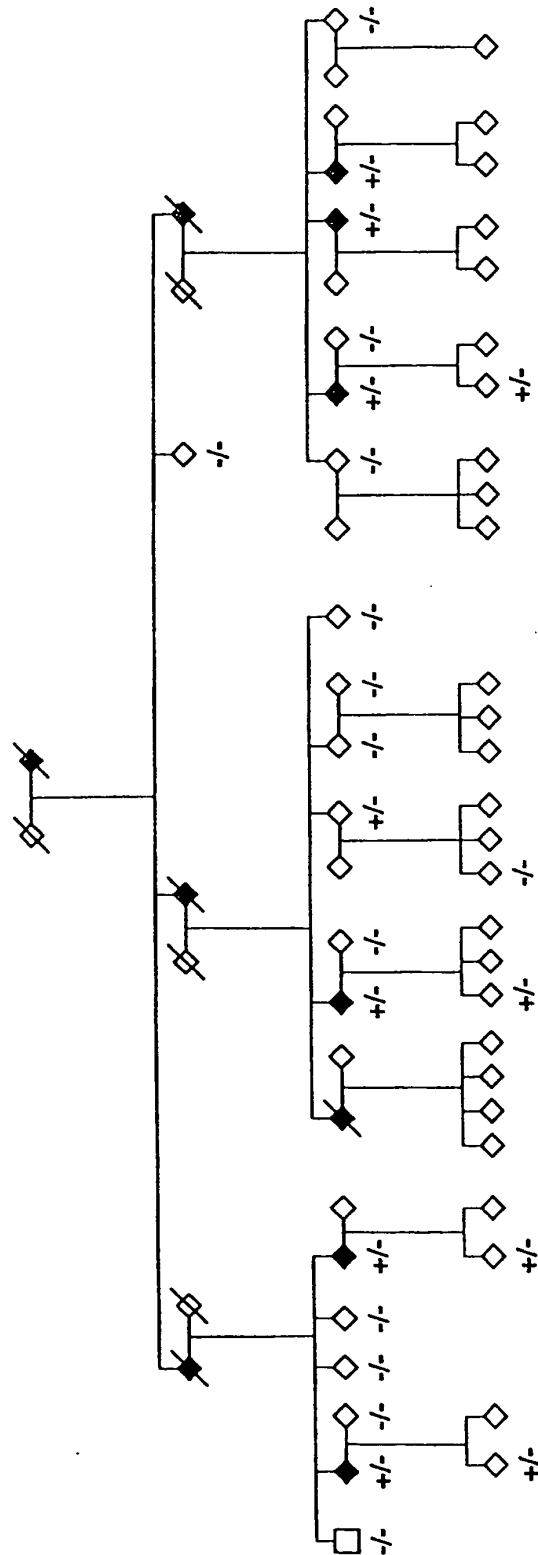


Fig. 3B

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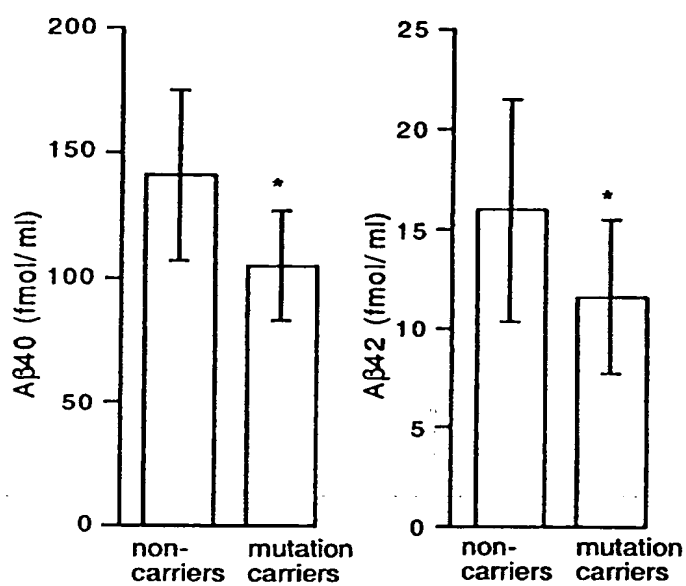


Fig. 4

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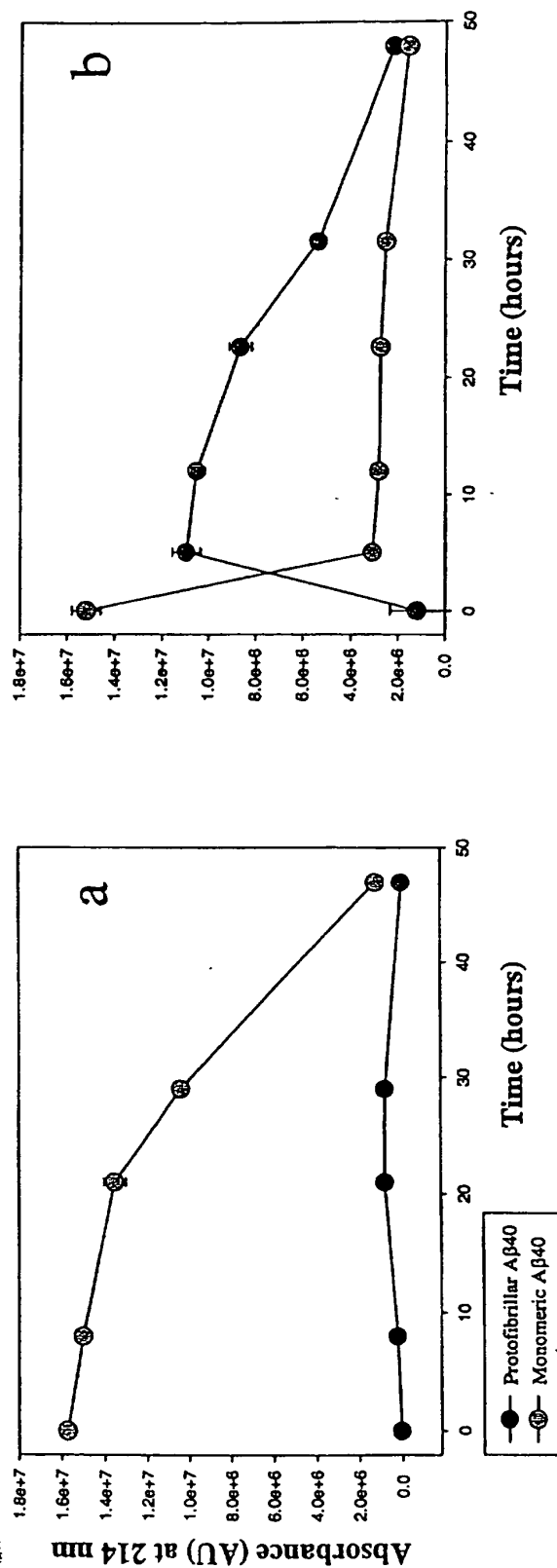


Fig. 5

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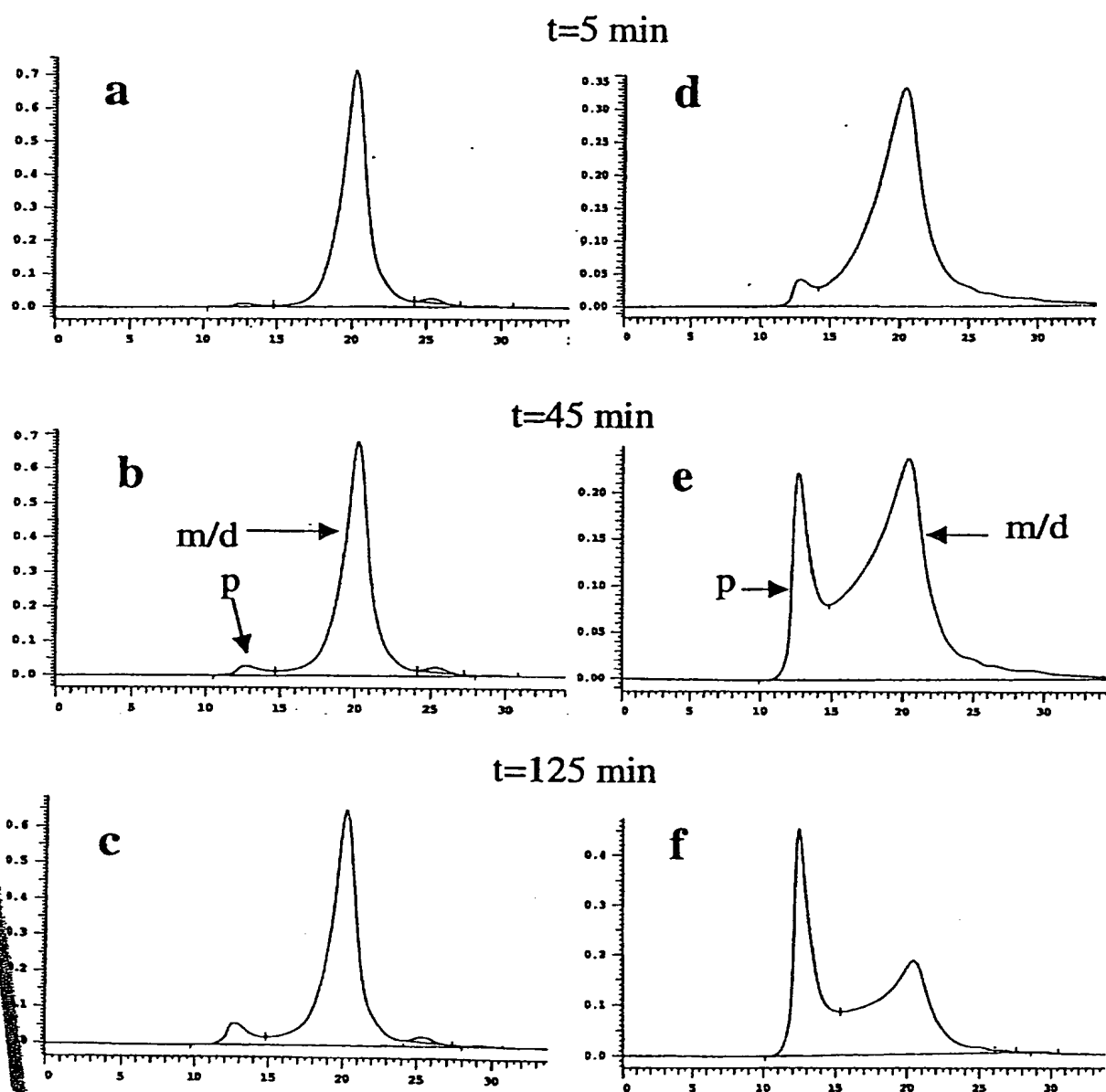


Fig. 6

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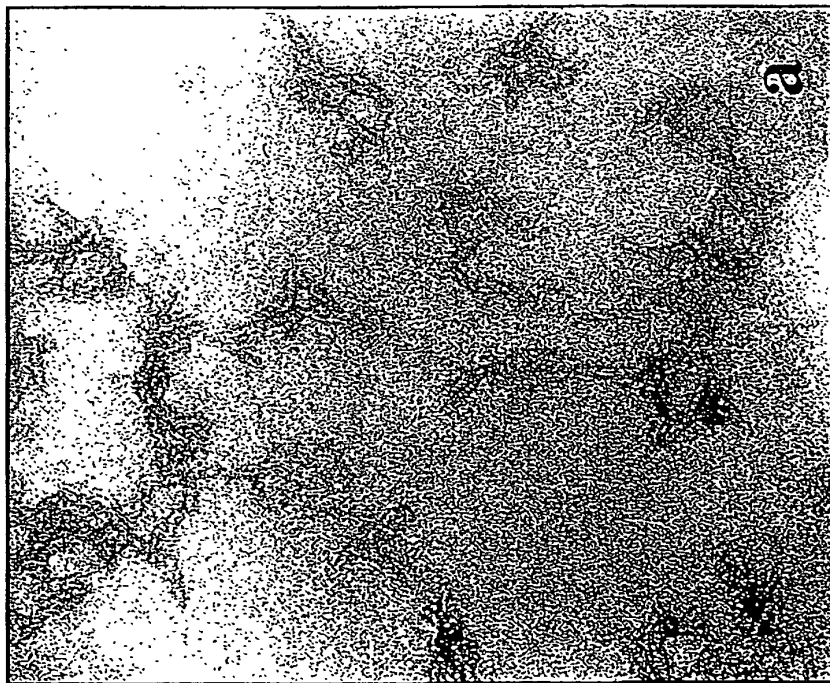
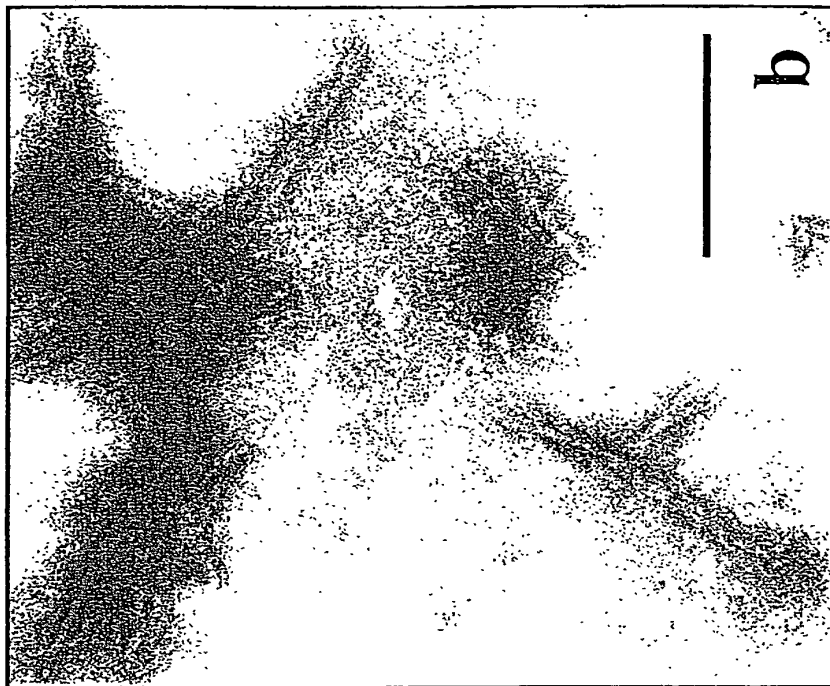


Fig. 7